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APHID TRANSMISSION OF POTATO LEAFROLL VIRUS [1]

H. R. MacCarthy [2]

SUMMARY

Non-infective, apterous, adult, single *Myzus persicae*, allowed to feed for a minimum of 5 days on newly-emerged potato plants infected with potato leafroll virus disease, transmitted the disease to about 70 per cent of *Physalis floridana* seedling indicator plants.

Eighty-three per cent of apterous *M. persicae* proved capable of transmitting the disease.

Alate *M. persicae*, fed for 5 days or longer on potato plants infected with the virus, were found to be capable of transmitting the virus for as long as they lived. A period on an immune host of up to 24 days, intervening between the acquisition and inoculation feedings, did not destroy the virus charge within the insect.

Distribution of infections chronologically by apterous and alate aphids, given a fresh indicator plant daily, was almost completely random. Most of the aphids remained infective until they died of old age.

The acquisition threshold period was found to be 2 hours. One aphid of 60 tested at this interval caused infection.

The inoculation threshold period was found to be 30 minutes. Five aphids of 120 tested at this interval caused infection.

The transmission threshold period was found to be 12 hours, comprising 4 hour acquisition and 8 hour inoculation feedings.

No infections were produced in 83 *P. floridana* seedling indicators individually inoculated by 40 adult, apterous *Macrosiphum solanifolii*.

Only 2 infections were produced in 170 *P. floridana* indicators inoculated by 180 adult, apterous *Myzus solani*. This species was somewhat toxic to the indicators.

Forty-one infections were produced in 176 *P. floridana* indicators, individually inoculated by 26 adult, apterous *Myzus ornatus*.

Aphis gossypii proved to be incapable of transmitting the virus.

Seven species of *Physalis* and 4 other plants were tested as indicators. These were: *P. floridana* and *P. angulata*, which were susceptible; *P. philadelphica*, which was slightly susceptible; *P. franchetii*, *P. pubescens*, *P. ixocarpa* and *P. heterophylla*, which were not susceptible. The latter was not acceptable to the chief vector. *Lycopersicon pinpinellifolium* was a symptomless carrier; *L. esculentum* was slightly susceptible; *Datura stramonium* and *D. stramonium* var. *tatula* were susceptible; a species of *Malva* was not susceptible. *P. floridana* proved to be an excellent indicator.

Ten species and/or varieties of *Brassica* proved to be nonsusceptible.

The latent period was found to vary widely with individual aphids, between 9½ and 120 hours.

Many fundamental relationships between potato leafroll virus (*Corium solani* H.), and its aphid vectors are still inadequately known, despite much research on the subject. The most important and costly virus disease of potatoes, it is the most difficult to avoid or eliminate. Recently, research has been stimulated by reports of newly discovered and quick-growing indicator plants. (7, 9).

The preeminence of the green peach aphid, *Myzus persicae* (Sulzer), as the chief vector of the virus, is firmly established in the literature. From the economic viewpoint, the problem of the vector relations of potato leafroll virus is virtually the relation of *M. persicae* with potato plants. It is not always the dominant species in the field, but may be replaced by the potato aphid *Macrosiphum solanifolii* (Ashmead) (= *gei* (Kalt.)), a much less efficient vector.

DEFINITIONS.—In this paper the terms used are those in common, current usage among virus workers. The naming of feeding and threshold periods follows Sylvester (17), with subsequent modifications (1, 14). Thus infection feeding is called acquisition feeding; test feeding is called inoculation feeding; and thresholds are called threshold periods.

To avoid repetition, the terms indicator, indicator plant, and test plant are used synonymously. Where one of these terms is used without further identification as to species, *Physalis floridana* Rydberg is meant in all cases.

Unless otherwise stated, the term aphid refers to an adult, wingless, green peach aphid, *Myzus persicae* (Sulzer). A noninfective aphid was one reared on Indian mustard, *Brassica juncea* Coss. This is an immune host plant, on which the stock colonies were maintained. An infective aphid was one that had fed on a diseased source plant for a minimum of 5 days.

An inoculation feeding by an individual infective aphid, with no further specification as to length, was 24 hours access time.

Except where otherwise stated, sources of virus were always potato plants of var. Netted Gem (= Russet Burbank).

MATERIALS AND METHODS.—*Materials.*—The cages used for inoculations with individual aphids on seedling test plants, consisted of open-ended glass tubes, made from 32 × 90 mm. shell vials, 1 end of which was closed with cotton cloth stretched and glued to

[1] Accepted for publication November 18, 1953.

Based on part of a thesis submitted to the Graduate Division of the University of California, Jan. 1953, in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Grateful acknowledgment is made to J. H. Freitag, E. S. Sylvester and M. W. Gardner.

[2] Assistant Entomologist, Field Crop Insect Section, Laboratory of Entomology, Kamloops, B.C., Canada.

the glass. The cages were pressed well into the screened top layer of soil in the 3-in. pots used, and proved eminently satisfactory.

Preliminary tests established the superiority as an indicator of *P. floridana* over other suitable test plants such as *P. angulata* L. or *Datura stramonium* L.; accordingly the conclusions are based on work with *P. floridana* unless otherwise stated. It is readily started from seed, is hardy, and is easily transplanted. Normally it is ready for transplanting 20 days from seeding.

Methods.—Disease readings were made 1, 2, and 3 weeks following inoculation. After about 4 weeks from planting, the indicators started to show the effects of crowded roots and undernourishment. With addition of fertilizer to the pots noninfected plants resumed normal growth, whereas infected plants responded scarcely at all. Distinct disease symptoms showed usually about 5 days or more after inoculation. A comparison was made of the above-ground, fresh weight of a random sample of 50 infected and 60 healthy test plants, from the same series, about 8 weeks from seeding, and 3–4 weeks from inoculation. Probability of the plants having been drawn from the same population was below 8 per cent.

The camel's-hair brush method was used to transfer aphids, when individuals were handled. The aphids were touched with the brush a few moments before they were lifted, in order to allow them to withdraw their stylets. In large-scale transfers, uniformity of access time was achieved by tapping the mustard leaves supporting stock colonies over a sheet of smooth, black metal, and sliding the mass of dislodged aphids off the metal and on to the virus source plant without further handling. No difficulty was experienced in inducing the aphids to feed on *P. floridana*, in spite of the fact that the plant is somewhat hairy.

Separate greenhouses were used to keep stock colonies and source plants apart from controls and test plants. The temperature in the house used for incubation varied between 70° F. and 75° F., with an extreme range between 65° F. and 89° F. The high and low temperatures obtained for very short periods only. This house was fumigated with "Nicofume" and sprayed with nicotine sulfate at regular intervals. All test plants were fumigated following inoculation and before being placed in the incubation house. *P. floridana* was seldom damaged by fumigation, but other *Physalis* spp. were somewhat susceptible. At no time did any of the numerous checks become infected by contamination. The chances of contamination affecting the results are therefore considered to be low.

Whenever possible a large number of seedling indicators was transplanted to the 3-in. pots at one time. Test plants were chosen from this stock for a given experiment on the basis of uniformity. A lapse of a few days between transplanting and inoculating gave seedlings a chance to become established, and avoided loss of experimental material from damping-off. The test plants were watered 1 or more times daily. All *Physalis* species tested appeared to be resistant to under- or over-watering, and to damping-off organisms.

RESULTS.—*Indicators tested.*—Thirteen species of *Physalis* have been tested and reported, in the hope of finding an indicator plant for potato leafroll virus, superior or alternative to *P. floridana.* Kirkpatrick[3] has reported on 10 of these, and the writer on 7 (Table 1).

The method used in this study was to inoculate young seedlings of the indicator under test, for 24 hours, with aphids reared on infected potato plants. After 10–15 days in which symptoms were observed, the plants were colonized for 5 days with noninfective aphids, which, in turn, were given an inoculation feeding on *P. floridana* seedlings. The tests were made with groups of replicated plants, and the whole procedure repeated up to 4 times if the indicator appeared promising. None was as useful as *P. floridana.*

Symptoms induced in *P. floridana* by potato leafroll virus have been adequately described (9), and are

TABLE 1.—*Combined results of tests of Physalis spp. as indicators for potato leafroll virus, made by Kirkpatrick and the writer*

Physalis sp.	Tested by	Result
floridana Ryd.	HCK[a] HRM[b]	Susceptible with symptoms
angulata L.	HCK HRM	Susceptible with symptoms
longifolia Nutt.	HCK	Susceptible with symptoms
lancefolia Roem.	HCK	Susceptible with symptoms
pruinosa L.	HCK	Susceptible with symptoms
peruviana L.	HCK	Susceptible without symptoms
alkekengi L.	HCK	Not susceptible
franchetii Hort.	HCK HRM	Not susceptible
pubescens L.	HCK HRM	Not susceptible
cotzmatl Moc. & Sesse	HCK	Not susceptible
ixocarpa Brot.	HRM	Not susceptible
philadelphica	HRM	Slightly susceptible; symptoms poor.
heterophylla Nees	HRM	Not susceptible, not acceptable to vectors.

[a] Initials HCK are those of H. C. Kirkpatrick.
[b] Initials HRM are those of the writer.

[3] H. C. Kirkpatrick, Wenatchee, Wash. Personal letter of October 8, 1951.

FIG. 1. Symptoms induced by potato leafroll virus in *Physalis floridana* plants at 3 stages. Left, healthy control plants; right, diseased plants. Top left: 1 week after inoculation by aphids. Top right: 2 weeks after. Bottom: 3 weeks after. All pictures to the same scale.

centimeters
1
2
3
4
5

clearly shown in Fig. 1. In every case the contrast between healthy and diseased plants was unmistakable.

Virus was recovered from *P. floridana* plants which had been infected directly from potato plants, and was re-transmitted by aphids to 160 healthy indicators in 7 experiments. Average transmission by single aphids between *P. floridana* plants was 21.3 ± 8.0 per cent. The virus titer in the indicator sources did not fall as these aged.

The effect of light on infected indicators was shown by loss of the diagnostic symptoms when the light intensity was reduced. The plants remained small, they provided a good source of inoculum, and the reproductive structures were inhibited although otherwise they appeared to be healthy. With restoration of full daylight, the characteristic symptoms returned within 2 weeks.

In groups of twenty, 100 seedlings of the dwarf tomato *Lycopersicon pimpinellifolium* (Jusl.) Mill., were inoculated by individual aphids. No symptoms resulted. A random sample of these was colonized with noninfective aphids which were then tested on *P. floridana* indicators. Two of the test plants showed potato leafroll virus disease symptoms, indicating that the dwarf tomato was a symptomless carrier. Since it showed no promise as a test plant no further experiments were made.

Commercial tomato seedlings (var. Marglobe), were inoculated individually with infective aphids. Of 60 experimental plants, only 6 showed transient, and 1 clear-cut symptoms (13). These were not checked back to *P. floridana*, nor tested further.

A possible weed reservoir of the virus was investigated in a common California weed of *Malva* sp. A procedure similar to that described, demonstrated that the plant was immune.

Ten different *Brassica* species, were tested as possible reservoirs of potato leafroll virus in the field. These were: cabbage, cauliflower, Chinese cabbage (var. Gai Choi, Pak Choi and Pe Tsai), Indian, black and yellow mustards, rape and rutabaga. Each day for 6 days, 5 infective aphids were placed singly on 5 seedlings of the plants named. For the same 6 days, the infection rate in identically treated *P. floridana* was 2/5,[4] 2/5, 3/5, 3/5, 2/5, 2/5, or 14/30. None of the inoculated Brassicas showed any symptoms. When colonized with noninfective aphids and checked back to *P. floridana*, no infections resulted.

Inocula tested.—Since potato leafroll virus has proved, so far, non-sap-transmissible and serologically inactive (4), and produces no inclusion bodies (6), there exists no method of estimating the amount of actual virus within a plant. Appraisal of the amount of phloem necrosis, using the phloroglucinol technique (11, 15), gives no useful quantitative measurement. Not only does the amount of necrosis differ with variety, but the disease can occur without any phloem necrosis (8). There was little point in trying to assess the effects of potato leafroll virus on the potato plant quantitatively, e.g., by measurement of leaf area, because the greenhouse, clay pot, and cage environments were so unsuitable and artificial that it was not possible to establish a norm. In view of the foregoing, it was thought that only 1 valid method remained for appraising source plants for virus titer, and of choosing that variety giving the most consistent results for further study. The method was to run a comparative study of varieties from different localities.

In this experiment, 5 indicator plants per day were inoculated by individual aphids from colonies on diseased plants of the varieties listed. The aphids were taken from all parts of the uniformly young source plants. The experiment ran for 6 days, giving 30 indicators per variety. Two species of test plants were included for comparative purposes, viz. *Datura stramonium* and *P. floridana*. Results are in Table 2.

Only transmission from potato was considered in the analysis. Table 2 shows that var. Netted Gem gave the highest and most consistent infection rate. It was therefore adopted as the virus source for the rest of the experiments. No strain effect was noticeable and all the infected indicators showed the same symptoms, indicating that a single strain of the virus was present.

Vectors tested.—All but 4 of the 15 vector species in 4 Orders, recorded by Heinze (5) are such inefficient vectors as to be of little or no importance. The exceptions are: *Myzus persicae*, *M. ornatus* Laing, *M. circumflexus* (Buckton) and *Myzodes ascalonicus* (Doncaster). Named stock colonies of some aphid vector species were available at the Experiment Station in Berkeley, and tests were made with the most promising of these: viz. *Myzus solani*, *M. ornatus*, *Macrosiphum solanifolii*, and *Aphis gossypii*. Colonies of *M. circumflexus* and *M. ascalonicus* were not available. In every case adult apterae were used, with the exceptions to be noted for *M. persicae*.

One hundred and eighty adult, apterous foxglove aphids, *M. solani* (Kalt.), were tested. Of these, 150 having fed for 5 days on a diseased potato plant, were placed on 30 indicators at the rate of 5 per plant, for 24 hours. These produced no infections. Further tests using 10 aphids fed similarly and placed individually on 10 indicators for 24 hours, again produced no infections. A further group of 20 single aphids was carried serially, using a fresh indicator plant every day for each surviving aphid until it died. Two infections, by different single aphids, were obtained. For practical purposes, 2 infections from 290 inoculations by 180 aphids on 170 test plants, might be considered negligible. It demonstrated, however, that transmission of the virus by the species was possible.

All 30 plants inoculated with 5 *M. solani* each showed mottling and twisting of the leaves, due to the toxic effect of feeding by this species. Recovery was rapid when the aphids had been removed. The other vectors tested appeared to be non-toxic.

[4] Numerator = plants infected; denominator = plants inoculated.

TABLE 2.—*Comparison of potato varieties and 2 indicator plants as sources of inoculum of potato leafroll virus*

Variety	Place of origin	No. infected per day per 5 plants						Inf./total	x per day	χ^2	P
Netted Gem	Prosser, Wash.	3	5	2	5	4	4	23/30[b]	3.83	1.78	NS
White Rose	Shafter, Calif.	0	0	3	2	3	4	12/29	2.00	7.00	NS
Russet	Shafter, Calif.	1	2	4	4	2	3	16/29	2.67	2.74	NS
Russet Burbank[a]	Aberdeen, Idaho	4	1	3	3	4	4	19/30	3.17	2.15	NS
USDA 41956	Geneva, N. Y.	0	1	2	4	4	4	15/30	2.50	6.20	NS
USDA 41956	Presque Isle, Me.	3	1	1	2	2	2	11/29	1.83	1.55	NS
Kennebec	Geneva, N. Y.	2	0	0	0	2	2	6/30	1.00	6.00	NS
Chippewa	Orono, Me.	3	3	0	3	1	2	12/30	2.00	4.00	NS
Netted Gem	Vancouver, B.C.	4	5	5	5	3	3	25/30	4.17	1.16	NS
Netted Gem	Pullman, Wash.	3	3	2	4	3	5	20/30	3.33	1.60	NS
D. stramonium	From seed	0	0	2	0	1	1	4/30	0.67	4.97	NS
P. floridana	From seed	4	0	0	0	2	2	8/30	1.33	10.02	NS

Sum of χ^2 for 10 potato treatments = 34.18, d.f. = 50, P = .28; denoting insignificant variation from uniformity of infection by days.
χ^2 for potato variety totals = 19.67, d.f. = 9, P = .02; denoting significant variation from uniformity of infection between varieties.

[a] Russet Burbank = Netted Gem var.
[b] Numerator = plants infected; denominator = plants inoculated.

Treated in a similar way, the ornate aphid, *Myzus ornatus*, proved to be a more efficient vector. Five infectives on each of 10 test plants, produced 8 infections. Of 26 individuals carried serially, 7 proved noninfective. Percentage transmission was 23.3, based on 41 infections from 176 individual inoculations.

A total of 83 indicators were inoculated by 40 potato aphids, *Macrosiphum solanifolii* (Ashm.). No infection resulted. Under caged conditions, colonies of this species built up very rapidly and were damaging to the potato source plants, but no toxic effect could be observed from 24-hour feedings on the indicators. It may be noted that K. M. Smith (16) could obtain no transmission of potato leafroll virus by this species, but Kirkpatrick and Ross (10) obtained 4 and 9 per cent respectively in 2 experiments using *Physalis* indicators.

A few individuals only of the melon aphid, *Aphis gossypii* Glover, survived on potato for the 5-day acquisition period. These failed to transmit potato leafroll virus when transferred to test plants.

Alate *Myzus persicae* were shown to be capable of transmitting the virus for as long as they lived. Some were reared on diseased potato plants, others were given the usual 5-day acquisition feeding. Once infective, the alates were held on *Brassica juncea* and kept separated from their apterous progeny. In 3 experiments, 5 alates were taken at 2-, 3-, and 4-day intervals, given 24 hours on individual indicators and then returned to the immune stock plant. When the number of survivors on the *B. juncea* fell below 5 at 23-28 days, the experiment was abandoned. The overall rate of transmission was 23/135, or 17 per cent. Evidently a period on an immune host, of up to 24 days, intervening between the acquisition and inoculation feedings, did not destroy the virus charge within the insect.

Acquisition time and infectivity.—Data in Table 3 were compiled from experiments conducted over a 6-month period. Not all these were directly concerned with timing of the acquisition feeding. Nonetheless the results may be compared, in that the variety of source plant was unchanged (with the exceptions noted); the vectors came from the same stock colonies; the treatment, except for the variable was uniform; and a number of the experiments were performed simultaneously. The data show that it was unusual (1/189) for aphids to pick up a virus charge in 8 hours or less, and that there was a fairly steady rise in infectivity up to about 100 hours, after which time there was no consistent gain. Klostermeyer (12) obtained similar results.

Threshold periods.—To arrive at an approximation of the threshold periods, a factorial experiment was designed (Table 4), to use small samples of 10 aphids for each of 49 combinations of acquisition and inoculation feedings, using the same source plant throughout. The entire series of 490 plants was inoculated

TABLE 3.—*Change of infectivity with acquisition time, using 1 apterous adult Myzus persicae per indicator plant and var. Netted Gem as inoculum sources*

Acquisition feed, in hr.	Result	Acquisition feed, in hr.	Result	Acquisition feed, in hr.	Result
1	0/50[a]	48	5/38[b]	120	13/14
2	1/29[c]	48	2/30[c]	120	6/14
2	0/50	56	2/30[d]	120	10/14
4	0/30[c]	74	17/30[d]	120	11/14
8	0/30[c]	96	14/30[c]	120	27/37
12	2/30[c]	96	20/60	144	9/24
12	3/50	120	16/39[b]	144	7/10
20	3/40	120	8/25	168	9/10
24	1/30[c]	120	9/12	216	7/10
40	14/53	120	10/14		

[a] Numerator = plants infected; denominator = plants inoculated.
[b] Source of inoculum was USDA seedling 41956.
[c] Data from factorial experiment, Table 4.
[d] Two aphids per indicator; total number of aphids = 907; total number of indicators 847.

within a week. All inoculations were made using 1 aphid per plant.

Other simultaneous experiments were then set up, to narrow and confirm the threshold periods demonstrated. These employed larger samples and used the marginal combinations of acquisition and inoculation feeding times (Table 5) as indicated by results of the earlier factorial experiment. In anticipation of a low probability of transmission, 4 infective aphids from the source plant were placed on each of 30 indicators at the 30-minute level of inoculation feeding. There were 5/30 infections, which would probably represent 5/120 in terms of infective aphids.

TABLE 4.—*Factorial experiment with small samples, showing combinations of acquisition and inoculation feeding times, 1 aphid per plant, 10 plants per combination*

Acquisition feed. in hr.	Inoculation feeding in hours						
	2	4	8	12	24	48	96
2	0	0	0	0	0	0	1
4	0	0	1[a]	0	0	0	0
8	0	0	0	0	0	0	0
12	0	0	0	0	0	1	1
24	0	0	0	0	0	0	1
48	0	0	0	0	0	0	2
96	1	3	6	3	5	3	6

[a] Figures = plants infected out of 10 inoculated.

The minimum value for the acquisition threshold period was 2 hours (Table 4). In the larger-scale, confirmatory test (Table 5), this value was not confirmed, nor did any infection result from inoculations by 50 aphids which had been given a 1-hour acquisition feeding.

The transmission threshold period, or minimum overall time taken to produce infection, was found to be 12 hours, comprising 4 hours acquisition and 8 hours inoculation feeding (Table 4). This marked lowering of the period from the 54 hours established by K. M. Smith (16), was supported by infections produced at 16 hours and 20 hours elapsed time (Table 5). More striking evidence is presented by Kirkpatrick and Ross (10), who were able to demonstrate a transmission threshold period of only 1½ hours. The latter used *P. floridana* as both source and indicator, and aphids in groups of 5.

TABLE 5.—*Tests for confirmation of small-sample factorial experiment (Table 4), showing combinations of acquisition and inoculation feeding times, 1 aphid per plant*

Acquisition feed. in hr.	Inoculation feeding in hours								
	½	1	2	4	8	12	24	48	96
1	[b]								0/50[a]
2									0/50
4									
8									
12				1/50	1/50	0/50	3/50		
24									
48									
96	5/30[c]	9/50	7/50						

[a] Numerator = plants infected; denominator = plants inoculated.

[b] Blank spaces represent tests not made. All tests made are shown.

[c] Four aphids per plant.

Retention period.—An experiment was conducted in 10 replications, to establish the length of time for which potato leafroll virus could be retained by *M. persicae*. Lots of 10 infective aphids were placed singly on indicator plants, and each individual given a fresh plant daily until it died. The large number of plants involved, required a good deal of greenhouse space, so that the experiments were run over a period of 6 months. The results are not, therefore, in the strictest sense comparable, but the sample is large and replicated enough to allow valid conclusions. The contrast between experiments, in numbers of plants infected, may be seen in the mean daily infection column in Table 6.

The insects and test plants were handled in uniform fashion, and the variations within and between treatments can be accounted for by the following circumstances: light and temperature conditions varied between November and May, and could have affected in some degree, the sources, the aphids and the indicators; the source plants may have differed physiologically, although of the same variety, with a single exception, and of approximately the same age. That the variation in transmission did arise largely from these factors is indicated by the last 4 of the 10 experiments, which were conducted in 2 pairs of simultaneous replications. Aphids from the same virus source plant were used for each concurrent pair of experiments. Results are closely comparable, both as to infection and survival (Table 6). For the infectivity of aphids Nos. 61-80, $\chi^2 = .006$, d.f. = 1, P = .94. For aphids Nos. 81-100, $\chi^2 = .009$, d.f. = 1, P = .92. By contrast, for the 6 earlier experiments, $\chi^2 = 12.59$, d.f. = 5, P = <.05, indicating a significant degree of variation from whatever cause. These data point up the extreme difficulty of exactly reproducing biological experimental conditions, in particular of keeping uniform sources of inoculum over a period of time.

In the calculation for the whole group, of χ^2, s and $\hat{Y}$, the figures for survival and infection were used only to the fifteenth day after leaving the source plant. At this time 18 aphids were still living, i.e., about 1/5 of the original sample of 100. For infection by days, statistical values are: $\chi^2 = 12.07$, d.f. = 14, P = .60, $\bar{y} = 46.1$ per cent. The variation thus could be accountable by chance alone.

Factors other than light and temperature contributed, to a smaller extent, to the wide individual variation in transmission. Some of these were: 1) The almost completely random distribution in time of daily infections by aphids. Linear regression of percentage of infection on time shows (Fig. 2) that there was, in the aggregate, a tendency to falling-off in percentage of infections caused ($\hat{Y}$ or regression coefficient = −1.009, t = 3.70, d.f. = 14, P = <.01). The trend

TABLE 6.—*Individual records of transmission and retention of potato leafroll virus, with survival, by 100 adult apterous Myzus persicae*

Date of Start	Aphid Serial Number	Plants infected out of total inoculated										x̄ daily infection	Geometric mean survival days
1951													
11/21	1-10[b]	2/3[a]	2/4	0/3	3/6	1/4	2/5	2/6	1/6	0/3	0/4	1.3	4.3
12/1	11-20	1/3	1/3	5/5	1/3	3/4	2/4	3/4	0/3	0/3	2/4	1.8	3.6
12/6	21-30	3/6	1/5	4/7	1/5	0/1	3/5	2/4	3/3	4/5	0/5	2.1	4.3
12/27	31-40	0/1	5/7	0/3	3/3	5/6	2/4	1/2	1/2	1/1	9/18	2.7	3.5
12/28	41-50	2/5	10/14	3/3	9/15	6/18	1/4	2/16	13/19	3/6	22/31	7.1	10.5
12/31	51-60	9/16	8/17	0/12	6/12	5/11	4/18	6/36	1/9	7/14	3/15	4.9	14.9
1952													
4/10	61-70	11/19	7/13	6/9	8/10	9/17	8/9	13/18	6/9	3/4	6/11	7.7	11.0
4/10	71-80	22/33	5/9	8/9	5/6	8/10	8/11	0/4	15/17	0/1	9/13	8.0	8.8
5/6	81-90	3/4	2/16	0/4	0/5	7/20	1/7	1/3	4/4	1/8	2/9	2.1	6.7
5/6	91-100	2/8	1/13	3/12	3/5	0/8	7/12	0/13	5/13	0/3	2/8	2.3	8.8

[a] Numerator = plants infected; denominator = total number of plants fed on by each aphid, and therefore total number of days insect survived.

[b] Individual aphid numbers run from left to right, within the groups of 10. No. 1 is in upper left-hand corner, No. 10 in upper right, No. 91 in lower left and No. 100 in lower right.

was too slight to be noticeable without a large sample, but does suggest, at least, that there was no multiplication of virus within the insects. 2) The differences in efficiency between individual aphids. These, however, were more apparent than real when subjected to analysis, indicating a common binomial distribution. The observed variance of the proportion of plants infected, (0.0283), did not differ significantly from the theoretical variance (0.0248). 3) The wide range of longevity. Of the 100 aphids started, 4 died within 24 hours, 2 more during the next day. At the other extreme were 3 which lived longer than 30 days. Probability of a homogeneous population with respect to longevity, was below 4 per cent.

Analysis of variance of the figures, shows that the variation in the number of days of life, or plants inoculated per aphid, was significantly greater between than within the replicates of 10 aphids. The logarithmic transformation was used: $y = \log_{10}(x + 1)$, where x = number of plants inoculated per aphid.

At least 83 of the 100 aphids proved to be capable of transmitting the virus. Most of those failing to cause infection died within 5 days, but 3 lived for 8, 12 and 13 days respectively. The evidence from the 2 retention experiments, with apterous and alate *M. persicae*, indicates that virtually any green peach aphid, feeding for 5 days or longer on a potato plant having potato leafroll virus, can pick up a charge sufficient to render it potentially infective for life.

DISCUSSION.—The term "latent period" is now generally assumed to mean a definite period, elapsing between the feeding of an insect on an infected plant, and the time when the same insect can infect another healthy plant, but not counting the acquisition and inoculation threshold periods. Smith (16) suggested that the latent period for potato leafroll virus in *M. persicae* was approximately 46 hours. He found that a noninfective aphid could pick up the virus in 6 hours and transmit it in 2 hours. The whole process was not completed in 8 hours, but a minimum of 54 hours elapsed time was necessary. He pointed out that it was unlikely that these were fixed times.

From the evidence presented here, an individual latent period would appear to obtain, varying with the individual insect within wide limits. At the lower limit it is suggested that the minimum time might eventually be no longer than that necessary for the vector to find the phloem, once in the source and once in the indicator. Given large enough samples and optimum conditions, there appears to be no reason why, in a few cases of fortuitous combination, the gap between acquisition and inoculation should not be eliminated. Since the work for this paper was done,

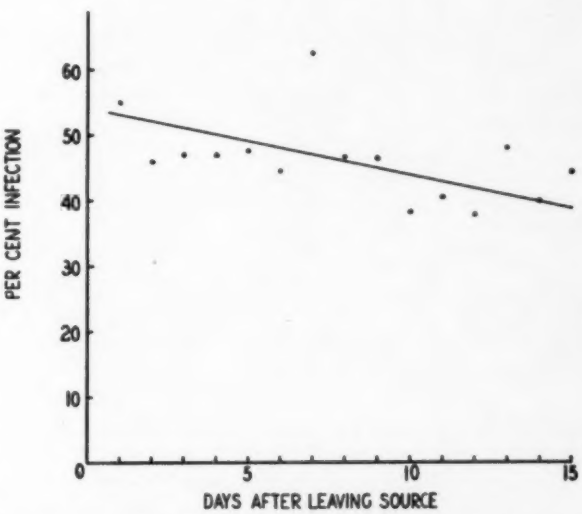

FIG. 2. Retention and percentage transmission of potato leafroll virus by *Myzus persicae*. Assumed linear regression is shown. $\hat{Y} = -1.009$, $t = 3.70$, d.f. = 14, $P = <0.01$.

Kirkpatrick and Ross (10) have approached this by demonstrating a latent period of less than 1½ hours, using aphids in groups of 5, and Klostermeyer (12) a total transmission time of 20 minutes. But the fact remains that in the vast majority of transmissions of the virus, there is a latent period, albeit an indefinite one, and good evidence exists to support any of the hypotheses so far advanced to account for it (2, 3, 11).

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STEMPHYLIUM LEAF SPOT OF LETTUCE [1]

B. Padhi and William C. Snyder

SUMMARY

A leaf spot disease has been observed on all varieties of lettuce, *Lactuca sativa* L., grown in or near Colma, a coastal district of California. The spots are brown, circular, and zonate, often they show a necrotic light-colored spot in the middle. The disease is most prevalent during the rainy winter months.

The incitant is a form of *Stemphylium botryosum* Wallr., imperfect stage of *Pleospora herbarum* (Pers.) Rabenh.

Spores of the fungus germinate readily on the leaf, and germ tubes penetrate into the tissues through the stomatal openings. Invading mycelium kills the host tissue in advance of fungus growth, but ordinarily its growth in a living leaf is limited. The fungus resumes its development when attacked leaves fall to the soil; the fungus then grows saprophytically throughout the leaf. Conidia and the ascigerous stage may be produced on disease refuse.

Several isolates of *S. botryosum* have been obtained from different habitats and localities. They have been found to be morphologically similar. All the isolates have been inoculated on lettuce, but only those originally isolated from lettuce leaf spots and an isolate from decaying artichoke buds in the same county have been found to be pathogenic with the production of leaf spots.

The lettuce pathogen has been inoculated on onion, carrot, bean, tomato, and sweet potato but found non-pathogenic to them.

Because of its selective pathogenicity for lettuce, the pathogen is named *S. botryosum* f. *lactucum*, forma nova, the imperfect stage of *P. herbarum* f. *lactucum*, forma nova, pathogenic on, and causing a leaf spot disease of lettuce, *Lactuca sativa*.

A leaf spot of lettuce, *Lactuca sativa* L., has been observed for at least 20 years in the Colma vegetable-growing district of San Mateo County, California. Although various types of lettuce are grown there the year around, the disease is most prevalent and causes the greatest damage during the rainy winter season.

Collections of diseased plants have been made from time to time, and the spots cultured. These cultures have yielded such ubiquitous fungi as species of *Stemphylium*, *Alternaria*, and *Cladosporium*. Because of their common occurrence as secondary organisms, it had been assumed that species of these genera were not involved as primary incitants. Consequently, a study of the disease was undertaken in the spring of 1950.

Occurrence elsewhere.—Several leaf spot diseases of lettuce have been reported from the United States (8) which in their symptoms bear some resemblances to the Colma leaf spot. The pathogens, *Septoria lactucae* Pass., *Marssonina panattoniana* (Berl) Mang., and *Cercospora longissima* Sacc., however, were never found associated with nor isolated from the specimens of the Colma leaf spot disease.

Several workers in other parts of the world have linked *Stemphylium* with the appearance of a leaf spot of lettuce. Ogilvie and Mulligan in 1931 (10) reported a leaf spot disease of lettuce as common in the West of England. The disease was found to be incited by *Pleospora herbarum* (Pers.) Rabenh., with an imperfect form in *Macrosporium sarcinula* Berk. The latter binominal is now regarded (14) as a synonym of *Stemphylium botryosum* Wallr. Dippenaar in 1939 (3) described a fairly serious leaf spot disease affecting lettuce grown during the winter at Cape Flats in South Africa. He identified the pathogen to be the same as that described by Ogilvie and Mulligan. Neergaard in 1945 (9) cites lettuce as a host for several species of *Stemphylium* and *Alternaria*. The Index of Plant Diseases in the United States issued in 1950 (8, p. 195) lists *Stemphylium* and *Alternaria* as incitants of secondary leaf spots of lettuce in a few states but there is neither mention of a primary leaf spot incited by these fungi in the United States, nor any reference to the occurrence of secondary leaf spots in California. In 1951, Pettinari (11) in Italy and Goossens (5) in Holland have reported *Stemphylium* leaf spots of lettuce. It is evident that a *Stemphylium* leaf spot similar to, or identical with, the Colma leaf spot has been established in many countries, but that such a disease has not been recognized for the United States.

Materials and methods.—Lettuce leaf spots were collected from time to time from Colma. All isolation work was carried out on sterile pea-straw agar plates, as described by Snyder and Hansen (12). Bits of leaf and petiole tissue, bearing an entire diseased spot, were cut out, washed in sterile water and placed on straw agar plates. Some of the plates were incubated out-of-doors, and others inside the laboratory. Abundant fungus conidia were formed in all cases within 7–8 days. Single spore transfers were made from these plates following the technique described by Hansen and Smith (7), and pure cultures of the fungi were obtained.

Lettuce plants grown in 4-in. pots both in the greenhouse and out-of-doors were inoculated by spraying them with spore suspensions from 20-day-old pure cultures and incubated in moist chambers.

The method of humidity control suggested by Stevens (13) was utilized for spore germination tests. A saturated solution of chloral hydrate was used to clear infected tissues for microscopic examination of the path of penetration, and a 0.02 per cent solution of cotton blue in lactophenol employed for staining and mounting.

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SYMPTOMOLOGY.—The first symptoms of the disease are visible as minute, pale, water-soaked specks. About 2 days after their first appearance the spots become conspicuous and brown. Usually, by the end of 2 weeks the leaf spots attain their maximum size. They are definitely circular in outline, chestnut-brown in color, and often zonate (Fig. 1A, B). The spots are sunken in the middle because of necrosis and collapse of the tissues. A single infection ordinarily does not spread widely within the leaf, but under favorable conditions, the fungus may advance rapidly throughout the tissues and cause scorching and, finally, death of the entire leaf (Fig. 1C). Sometimes the centers of larger spots drop out leaving a small hole in the middle of the spot. Under wet conditions mycelium may develop on the surface of the leaf tissue, more commonly on the lower side, and produce blackish conidia which give the spots a dark appearance. The symptoms in general are those described elsewhere (3, 5, 10, 11). Plants badly infected are unmarketable.

ETIOLOGY.—From more than 60 individual leaf spots, sampled at random from specimens collected at different places and times, and plated on agar media, only a very few fungi were isolated. *Stemphylium ilicis* Tengwell., *Alternaria tenuis* Wallroth, and *S. botryosum* were most consistently isolated and are listed in the order of their increasing frequency. Though the first 2 fungi were isolated frequently, they were seldom found alone. Usually the last one was isolated from the same spots. In more than half the cases only this fungus was isolated. Inoculation tests were performed with all isolates, but symptoms appeared and the inoculant isolated only from plants inoculated with *S. botryosum*, thus proving this fungus to be the pathogen.

In the preliminary tests lettuce plants were kept in the moist chamber for 24 hours, then sprayed with spore suspensions, and incubated for 48 hours. This procedure proved adequate for disease development; however, the moist chamber treatment prior to inoculations was found not to be essential. The incubation period following inoculations was reduced to 9 hours, without much alteration in degree of infection. In the wet, cool days of winter a large number of successful inoculations were performed out-of-doors, without the help of a moist chamber. It seems safe to assume that a single wet day is enough for the development of the disease, other factors being equal. Such days are very common during the winter in Colma.

Only stomatal penetration of leaves and petioles has been observed. This was true over a range of temperatures. Infection can occur on both surfaces of the leaf. In nature, especially on upright-lettuce varieties and on the Cos types, infection generally seems to take place through the underside of the leaf.

Lettuce plants of different ages have been inoculated and infections have been established readily on lower or outer leaves. But very young leaves have been difficult to infect. It seems that the stage of development of a leaf may have an important bearing on its susceptibility.

The spores, when germinating on a living leaf, send out 1 or 2, rarely more, germ tubes. The germ tube grows over the leaf surface and when it reaches a stoma, enters the substomatal cavity where it forms massive thick hyphae. From these hyphal masses, thin hyphae arise and spread into the leaf tissue (Fig. 1D). Local necrosis takes place in the vicinity of and in advance of such infection centers (Fig. 1E). The fungus is both inter- and intra-cellular following entrance into the leaf.

To determine the varietal susceptibility of lettuce, inoculations were made on All Year Round, Bibb, Bronze Beauty, Balloon Cos, Blond Long Standing, Continuity, Chicken, Imperial (847,456, and E4), New York 12, and White Seeded Simpson. All proved to be susceptible. Prize Head, White Boston, and Romaine, also, were prone to infection in field plantings.

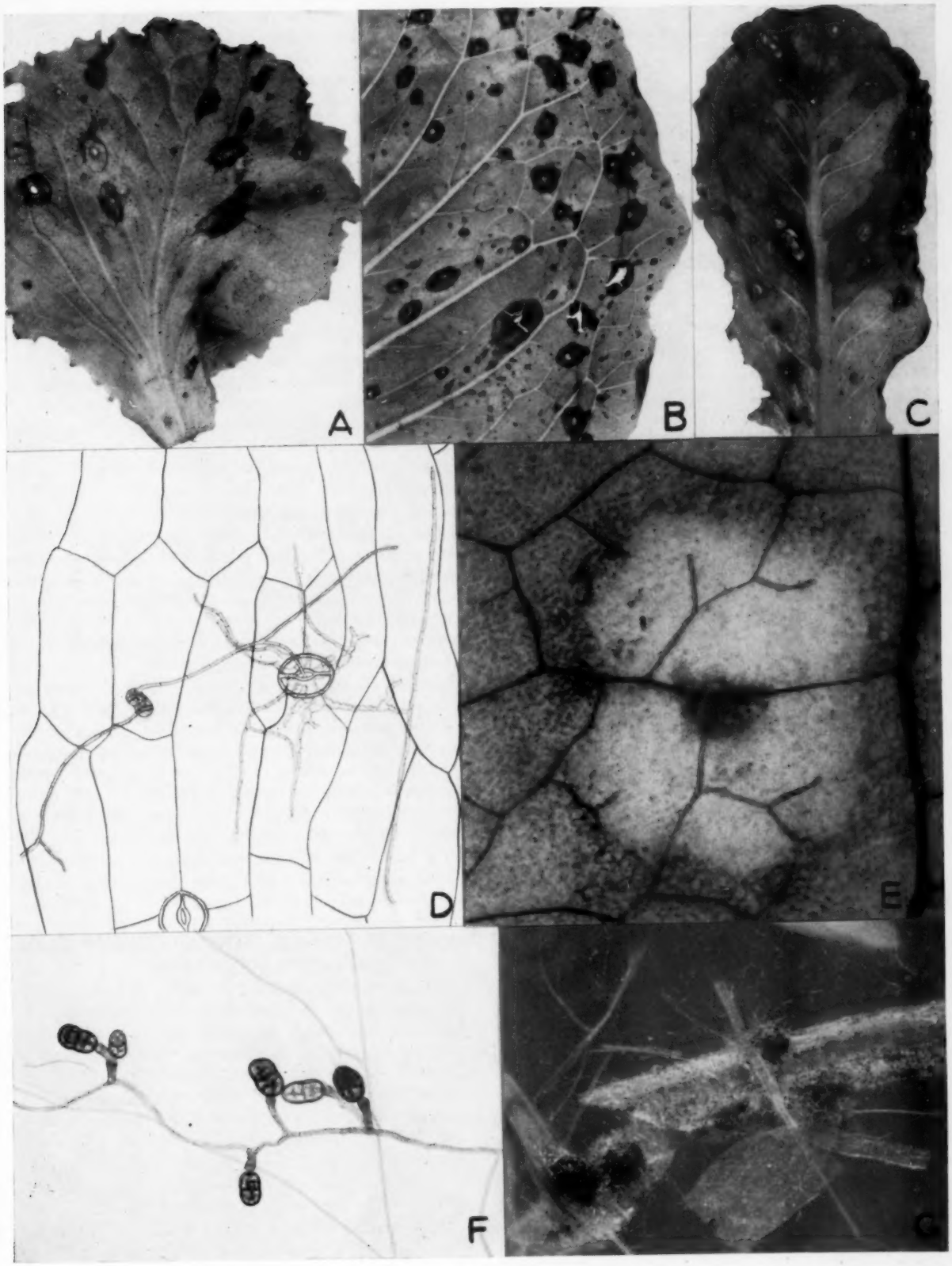
S. botryosum has been determined (4, 9) to be pathogenic on several other plants besides lettuce. To see if this particular strain of the fungus is also pathogenic towards those hosts, inoculations were made on onion (*Allium cepa* L.), carrot (*Daucus carota* L., var. *sativa* Dc), bean (*Phaseolus vulgaris* L.), tomato (*Lycopersicon esculentum* Mill), and sweet potato (*Ipomea batatas* (L) Lam). In no case did infection occur. It therefore seems clear that the fungus is selectively pathogenic toward lettuce.

PATHOGENICITY OF ISOLATES.—Since populations of *S. botryosum* would be expected to vary amongst themselves in morphological as well as physiological characteristics, several isolates of the species were collected from different substrates, in different localities, for comparison. These were inoculated to lettuce under conditions favorable for infection. The isolate from gladiolus was kindly supplied by Dr. J. G. Bald. Results are in Table 1.

Isolates obtained outside of San Mateo County, and therefore outside of the area in which the disease most commonly occurs, proved nonpathogenic to lettuce. In most cases these were probably non-pathogenic on their respective substrates. Except for a collection of the fungus from artichoke buds, in the San Mateo coastal area, only the isolates from lettuce were pathogenic to lettuce. It is suspected, but not proven, that

→

FIG. 1. A, B, and C) Natural infection of lettuce with Stemphylium leaf spot; A) on Imperial type head lettuce; B and C) on Romaine; note shot-hole effect in B, and scorching of leaf blade in C. D) Stomatal infection, showing massed hyphae (dotted lines) after entrance into the sub-stomatal cavity. E) Enlargement of leaf spot to show central dark area representing initial invasion area, surrounded by lighter zone of necrotic tissue killed in advance of fungus invasion. F) Conidia and conidiophores of *Stemphylium botryosum* f. *lactucae*. G) Perithecial stage, *Pleospora herbarum* f. *lactuae*, in straw agar culture; small specks on the straw are conidia.


A
B
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D
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TABLE 1.—*Pathogenicity of different isolates of S. botryosum to lettuce*

Source	Locality	County	Pathogenicity
Lettuce	Colma	San Mateo	+
Artichoke	Half Moon Bay	San Mateo	+
Alfalfa	Centerville	Alameda	—
Gladiolus	San Diego	San Diego	—
Sugar Beet	Salinas	Monterey	—
Tomato Stem	Salinas	Monterey	—
Carrot	Soledad	Monterey	—
Tomato Fruit	Brentwood	Contra Costa	—

the lettuce pathogen was growing saprophytically on injured areas of the artichoke buds, and was not a parasite.

GROWTH IN CULTURE.—All isolates, including those in Table 1, were similar in growth patterns and in morphology. Concordant variations served to link all isolates into continuous culture series. The morphology of the various clones was essentially in agreement with that extensively covered by Neergaard (9), but some additional comments may be made concerning conidial formation.

Although the manner in which conidia are borne on swollen heads of the conidiophores is considered a fairly constant characteristic, there was some variation in the morphology of the conidiophores. The lettuce isolates usually formed short conidiophores which bore mostly 1 and 2 conidia (Fig. 1F), whereas in the sugar beet isolate the first conidium was pushed aside and another conidiophore grew from the swollen head. In this way a large number of conidia could be borne on a sympodially formed conidiophore. However, both these processes have appeared at some time or another in all isolates.

Under cultural conditions the conidia not infrequently arose in short chains. Two and 3, rarely 4, conidia have been observed in chains on the isolates from lettuce, artichoke, alfalfa, and tomato fruit. The same phenomenon has been reported for the species of Stemphylium pathogenic to carrot (6).

The fungus grew well on such solid media as potato-dextrose, prune, oatmeal, cornmeal, yeast extract, and straw agars. It was also grown readily on autoclaved lettuce leaves, blotting paper, and soil. Only sparse surface growth, however, was obtained on Czapak's liquid medium. Straw agar was especially favorable for rapid and abundant formation of conidia.

Light seemed to have little effect upon the growth of fungus in culture and did not appear to be required for sporulation.

TEMPERATURE OPTIMUM FOR THE FUNGUS.—Cultures grown in triplicate on potato-dextrose agar, from 3-mm. discs of inoculum, were incubated at different controlled temperatures. At the end of 8 days the radial growth of each was measured. The minimum temperature for growth of the fungus was below 4°C., the optimum was between 22 and 28°C. and the maximum was above 34°C.

ASCIGEROUS STAGE.—All isolates, except the one from sugar beet, produced perithecial primordia in single spore cultures. Mature perithecia of *Pleospora herbarum* (Pers.) Rabenh., with viable ascospores, were obtained abundantly on a straw agar medium for the isolates from lettuce, artichoke, alfalfa, gladiolus, and tomato fruits. Thus these isolates were apparently sexually fertile and homothallic. Illustrations of the perithecia are in Figure 1G.

In parallel tests with ascospores and conidia, the same kind of results were obtained.

SPORE GERMINATION.—Since both conidia and ascospores may serve as inoculum for disease development, studies were undertaken to find out the conditions for their germination. Tests were performed on cellophane slides. These slides, with spores scattered on them, were placed on glass cylinders kept inside Kerr jars where an atmosphere of known relative humidity (R.H.) was maintained, as described previously. It was found that spores germinated only at and above a R.H. of 95 per cent. At saturation some spores began to germinate after 3 hours, but about 6 hours elapsed before most had germinated under laboratory temperatures of approximately 22°C.

Free water was not found necessary for the germination of spores nor was there any evidence to show that it promoted better germination. Spores germinated equally well both on a dry slide and in drops of water, when maintained in a saturated atmosphere. However, spores did not germinate well in water under a cover-glass, except at the edge of the cover-glass, indicating a positive oxygen requirement.

Almost all conidia, gathered from a fresh, week-old culture, germinated readily, while only about 50 per cent of those from a dry 6-month-old culture germinated. Ascospores collected from cultures which were a year old showed about 50 per cent germination, whereas conidia from the same culture gave only about 10 per cent germination. Ascospores from plates 15 months old showed about 20 per cent germination, and those 18 months old, less than 1 per cent germination.

Spore germination takes place over a range of temperatures from about 4°C. to about 37°C. Germination begins after 3 hours at and between 22°C. and 25°C. in a saturated humidity, but is delayed increasingly at lower and at higher temperatures.

Spores germinated equally well on dry glass slides in water drops, in water drops containing bits of boiled lettuce tissue, on water agar, and on potato-dextrose agar, when incubated at 25°C. in a R.H. of 100 per cent. Neither the difference in kind of surface on which the spores were placed, nor the presence or absence of nutrients materially affected germination within the limits of these tests.

EPIPHYTOLOGY.—The climate of Colma is very similar to that recorded for San Francisco, since the weather station lies just south of the latter city limit. The average annual temperature (1) is 56.2°F. Within the 5-year period 1946–1950, the minimum temperature was 32°F. and the maximum 92°F. An annual average

precipitation of 17.17 in. is recorded for the same area. Of this total, more than 90 per cent of the rainfall occurs from October to March.

Lettuce is grown extensively in this region for the local markets of the San Francisco Bay area, and practically throughout the year. Overhead irrigation is resorted to during rain-free periods. Varieties most commonly grown include the loose leaf lettuces, such as Black Seeded Simpson and Prize Head, the Cos types, and such heading types as Big Boston or White Boston.

The disease is most prevalent in late fall, winter, and early spring, when the weather is both cool and wet, and only occasionally at other times of the year. There appears to be a direct correlation between the climatological data, the experimentally determined requirements for spore germination, and the serious seasonal incidence of the disease in the field.

Since the temperature inside the leaf may be higher than the outside air temperature (2, chap. 11), the fungus may find more favorable temperature conditions in the leaf on cool days than might be indicated by prevailing daily air temperatures.

Reports of Stemphylium leaf spot disease in other parts of the world have come from regions in which lettuce has been grown under climatological or coastal situations similar to those in the Colma area.

Nomenclature of the pathogen.—Neergaard in 1945 (9) summed up the accumulated information on pathogenicity in *S. botryosum*. He states: "In the literature numerous reports have been made from nearly every part of the world about the occurrence of the fungus on hundreds of plant species." And: "*S. botryosum* is a pronounced ubiquitous facultative parasite, which most often is encountered on dead or greatly weakened plant elements; less frequently it may be demonstrated as the direct cause of morbid phenomena." He did not mention any specialization in the species.

S. botryosum is a cosmopolitan species. It is widely distributed and successfully established in many different kinds of environment on numerous substrates. Apparently there has been some specialization, and some populations have developed as pathogens. Such appears to be the case with isolates from lettuce. Although these isolates do not differ significantly in morphology from other isolates of the species, they are shown to be specialized in respect to their pathogenicity for lettuce leaves. It seems probable that such specialization may be common in the species.

Because the lettuce fungus is biologically specialized as to host, and is of economic importance, it seems desirable to indicate this specialization in the nomenclature of the pathogen. Consequently, it is proposed to designate the incitant of the Stemphylium leaf spot of lettuce as a distinctive form of the species, namely, as *Stemphylium botryosum* f. *lactucum*, forma nova, the imperfect stage of *Pleospora herbarum* f. *lactucum*, forma nova; pathogenic to the above-ground parts of the species, *Lactuca sativa*.

Discussion.—This disease has not threatened the lettuce industry of California nor does it appear likely to do so. Although now recognized as an old disease, it has remained localized. Its total effect is ordinarily not serious even where the disease occurs. However, the brown spots on the lettuce leaves reduce the quality and the market value of the crop. When conditions favorable for the disease have occurred, damage to the crop has been so extensive as to require the total abandonment of entire fields at harvest time. Such crop losses have been observed in South Africa (3), and in the Colma area during the wet winter of 1951-52.

No attempts at disease control have been made in the Colma area. Dippenaar (3) in attempting to control the disease in South Africa, tried Verderame (copper oxychloride) and copper lime mixtures as dusts, and Verderame and Bordeaux mixtures as sprays. Using 4–5 treatments, at 8–10 day intervals, he was able to obtain good control. This offers a means of disease control where the economics of the crop would justify such measures.

Where the use of fungicides is not feasible, improved crop sanitation may reduce disease incidence. The fungus lives over from crop to crop on dead lettuce leaves on the soil, and under favorable weather conditions produces the spores which are disseminated to the new crop. The disease refuse from an infected crop, therefore, should be turned under the soil to prevent release of spores into the air, and to hasten its decay.

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X-DISEASE VIRUS: I. HOST RANGE AND PATHOGENESIS IN CHOKECHERRY[1]

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SUMMARY

Host range and pathogenesis of the X-disease virus on chokecherry were studied in the field and in the greenhouse. Three strains of the virus were erected on the basis of differences in symptomatology in chokecherry. The virus was found in both aerial and subterranean portions of diseased chokecherries during the growing season and in aerial portions during dormancy. It was readily transmitted from dormant buds of diseased chokecherry after 35 days' storage at 34–36° F. It moved relatively slowly through chokecherry from the inoculation sites and was not transmitted from buds of symptomless branches taken 12–18 in. away from branches exhibiting symptoms. Some large chokecherry trees failed to exhibit symptoms in all portions 23 months after inoculation.

Combinations of the X-disease virus with necrotic ring spot virus, prune dwarf virus, sour cherry yellows virus, or green ring mottle virus failed to demonstrate evidence of cross-protection or of alteration of symptom expression of X-disease. Wild chokecherries infected with both X-disease and necrotic ring spot viruses were commonly found in the field.

Transmission of X-disease to 15 species of Prunus not hitherto recorded as hosts was accomplished. These were *P. domestica* var. Italian Prune, *P. dunbari*, *P. glandulosa*, *P. lannesiana*, *P. maacki*, *P. mahaleb*, *P. maritima*, *P. newporti*, *P. padus*, *P. pensylvanica*, *P. pumila*, *p. salicina* vars. Abundance and Burbank, *P. serrulata* var. Kwanzan, *P. sieboldi* var. Takasago, *P. spinosa*, and *P. tomentosa*. Previous reports of transmission of the virus to *P. americana*, *P. avium*, *P. besseyi*, *P. cerasus*, and *P. japonica* were confirmed.

No symptoms of X-disease were produced on *P. serotina* and attempts to recover the virus from inoculated trees to chokecherry were negative.

Stoddard (6) reported the effects of X-disease in peach and made a limited survey of the host range of the causal virus in the genus Prunus. The disease was found in the field on chokecherry (*Prunus virginiana* L.), peach (*P. persica* (L.) Batsch), and nectarine (*P. persica* var. *nectarina* (Ait.) Maxim.) (6). Additional hosts, sour cherry (*P. cerasus* L.) and sweet cherry (*P. avium* L.), were later reported (2, 7). In addition to these hosts (3, 4, 8), apricot (*P. armeniaca* L.), Big Mack plum (probably *P. insititia* L.), and a Duarte-Satsuma plum hybrid (probably a complex cross of *P. munsoniana* Wight & Hedr. with *P. salicina* Lindl.) have been found infected by the Western X-disease virus (5).

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Experimental transmission of the X-disease virus to Hansen bush cherry (*P. besseyi* Bailey), wildgoose plum (*P. munsoniana*), almond (*P. amygdalus* Batsch), American plum (*P. americana* Marsh.), and Chinese bushcherry (*P. japonica* Thunb.) was reported by Stoddard (6). With the exception of *P. americana*, which carried the virus without evident symptom expression, all the experimentally infected hosts exhibited some degree of chlorosis or anthocyanosis accompanied by dwarfing of the terminal growth.

Palmiter and Hildebrand (1) tested 45 varieties of peach and found all susceptible to X-disease. Stoddard (6) was unable to recover the virus from experimentally inoculated trees of black cherry (*P. serotina* Ehrh.) and beach plum (*P. maritima* Marsh.) and concluded that these species are immune.

The present study was initiated to contribute to the knowledge of the host range of the X-disease virus in the genus Prunus and to determine the pathogenesis of X-disease on chokecherry.

MATERIALS AND METHODS.—X-disease virus was ob-

tained from naturally infected wild chokecherries growing either in Door County, Wisconsin, or in Livingston County, New York. Principal sources of diseased budwood are characterized in Table 1.

Inoculations were made by budding or grafting. Because transmission of the virus was likely to be erratic when only a single bud was used for inoculation, 3 or more diseased buds were used whenever possible.

Inoculations made at any time during the growing season resulted in successful transmission of the virus, but unless they were made at budbreak or immediately afterward, symptoms were seldom expressed in the current growing season.

Host range determinations were made almost entirely with small potted trees in the greenhouse. Trees customarily were forced into growth in late January, inoculated as growth commenced, and observed until late May. At the latter time, the potted trees were moved to the field and inserted in soil to a depth sufficient to cover the tops of the pots with about an inch of soil. The trees were lifted in early November and placed in deep cold frames to complete the necessary cold requirement. They were moved into the greenhouse again the following January and forced anew for further observation.

Trees failing to develop symptoms of X-disease during 2 growing seasons following inoculation were held for further observations until they had been indexed on a suitable host for determination of presence of the X-disease virus.

At least 8 trees of each species of Prunus tested were inoculated with the X-disease virus, and each inoculated tree was indexed on 2 determinative hosts. In most cases, chokecherry was used as the indexing host, but both peach and *P. glandulosa* Thunb. were occasionally employed.

Studies of the pathogenesis of X-disease on chokecherry were made in the field as well as in the greenhouse. Large 6–8–year-old trees in a nursery in Door County, Wisconsin, were used in the field studies. No instances of natural spread of X-disease to plants in the nursery had been observed prior to experimental introduction of the virus in 1948, but 2 noninoculated trees became diseased in late 1949.

SYMPTOM EXPRESSION OF X-DISEASE.—*On chokecherry.*—The syndrome of X-disease on chokecherry was described by Stoddard (6) and Palmiter and Hildebrand (1). An undescribed symptom, consisting of a delay in foliation, was observed in both naturally and experimentally infected chokecherries. With all strains of the X-disease virus employed, the delay was slight but distinct. It was very perceptible in large trees experimentally infected, since these trees showed delay in foliation in the inoculated branches only. The delay was transitory and had become almost indiscernible 2 weeks after growth began. Foliar chlorosis and anthocyanosis did not appear until 6–8 weeks after growth had commenced.

TABLE 1.—*Principal field sources of the X-disease virus used in the greenhouse and in the field, 1948-1951*

Chokecherry source tree[a]	Type of symptoms expressed on source tree[b]	Presence or absence of necrotic ring spot virus
F-3	Anthocyanotic	Absent
F-8	do.	Undetermined
F-13D	do.	do.
F-35	do.	do.
F-8A	Proliferated	Present
F-13A	do.	do.
F-13B	do.	do.
F-13C	do.	do.
F-22	do.	do.
F-13	Chlorotic	Absent
F-47	do.	Present
F-43	Non-rosetted	Absent
XD-1	Proliferated	do.
XD-2	do.	Present
XD-3	Anthocyanotic	Absent
XD-4	do.	Present

[a] Trees labeled "F-" were located in Door County, Wisconsin; those labeled "XD-" were located in Livingston County, New York.
[b] See text for description of symptoms.

Palmiter and Hildebrand (1) reported variations in the presence and degree of anthocyanosis and chlorosis in infected chokecherries. Similar variations were encountered in the present study. With the exception of a proliferating symptom that was not encountered previously, these discrete variations were reproduced in experimentally infected chokecherries, the symptoms obtained in inoculated plants being of the same type as shown by the individual trees from which inoculum was obtained (Table 1).

1) *Anthocyanotic type.*—Foliage of infected chokecherries became intensely anthocyanotic following an initial chlorosis. Pronounced rosetting of the terminals (Fig. 1) was apparent by the second year of infection and became more pronounced in succeeding years. Fruits were sparse or lacking on trees in the second year of infection and those that were set did not mature normally. In the third and fourth years of the disease, trees were characterized by sparse foliation, almost complete absence of new terminal growth, and many dead or dying branches. Wild plants seldom survived the fourth year of the disease.

In many trees an additional symptom occurred. Terminal and axillary buds of the diseased branches produced a proliferating growth (Figs. 2, 3, 4,) that ultimately resulted in the formation of a pronounced witches'-broom. Leaves of the proliferated growth were initially bright yellow-green even though produced from buds of branches whose foliage was markedly anthocyanotic. Basal leaves of the proliferated growth ultimately became anthocyanotic, but, in many cases, the terminal leaves remained green until the end of the growing season. The proliferated symptom could not be consistently produced in trees inoculated from proliferated source trees, and also commonly appeared in trees inoculated from anthocyanotic source trees that did not proliferate. This symptom is

FIGS. 1–4. Fig. 1. Marked terminal rosetting resulting from infection with an anthocyanotic strain of X-disease virus on chokecherry in the greenhouse (right). Healthy plant on the left.—Fig. 2. Proliferation and anthocyanosis of *Prunus besseyi* infected by a proliferating source of X-disease virus.—Fig. 3. Proliferation of experimentally infected chokecherry in the field during late July.—Fig. 4. Proliferation of X-diseased chokecherry in the greenhouse following infection by an anthocyanotic strain of the virus.

not considered to be the expression of a distinct strain of the X-disease virus, but rather is a symptom produced by the anthocyanotic strain in plants of high vigor.

2) *Chlorotic type.*—The initial chlorosis of the foliage was not followed by intense anthocyanosis, but the foliage became bronze-yellow with a slight reddish cast and remained so until leaf abscission in the autumn. Fruit symptoms were like those of the anthocyanotic type. Terminal rosetting and failure to set fruits appeared in the second season of infection, but infected plants did not decline as rapidly as those with the anthocyanotic type of disease. The original source tree (F-13), which had been diseased at least 2 years when surveyed in 1948, was still alive in 1952.

3) *Non-rosetted type.*—Chlorosis followed by anthocyanosis appeared, but occurred approximately 2–3 weeks after the corresponding symptoms had appeared in trees affected with the anthocyanotic type of disease. Terminal elongation was only slightly reduced in the second and succeeding years of infection. Fruits borne on the original tree (F-43) were normal in coloration in 1948 and 1949 but were sparse and very sparse, respectively.

The anthocyanotic and chlorotic strains of the X-disease virus were readily transmissible, but inoculations with the non-rosetted strain resulted in very low percentages of transmission.

On other Prunus species.—Symptom expression of X-disease in the various species of Prunus experimentally infected fell into 3 characteristic groups.

1) *Group I.*—Characterized by expression of chlorosis or anthocyanosis accompanied by degrees of terminal rosetting and ultimate marked decline or death. Species evidencing this syndrome were *P. besseyi, P. dunbari* Rehd.,[2] *P. glandulosa, P. japonica,*[3] *P. lannesiana* Wils., *P. maacki* Rupr., *P. maritima, P. padus* L., *P. persica, P. pumila* L., *P. salicina* vars. Abundance and Burbank, *P. tomentosa* Thunb., and *P. virginiana.*

2) *Group II.*—Characterized by marked terminal rosetting and dwarfing unaccompanied by chlorosis or anthocyanosis and less precipitant decline than in Group I. Slight malformations of the leaf laminae or rugosity were commonly evident. Species evidencing this syndrome were *P. cerasus* var. Montmorency (on mahaleb understock) and *P. cerasus* seedlings, *P. avium* var. Black Tartarian, *P. serrulata* Lindl. var. Kwanzan,[4], and *P. sieboldi* Wittm. var. Takasago.[4]

3) *Group III.*—Characterized by lack of symptoms, but the X-disease virus was recovered by indexing. Species in this group were *P. americana,*[4] *P. domestica* L. var. Italian Prune,[4] *P. mahaleb* L.,[4] *P. newporti* Hort.,[5] *P. pensylvanica* L.,[4] and *P. spinosa* L.

P. serotina did not exhibit symptoms following inoculation nor was the X-disease virus recovered to chokecherry from any of the 16 trees of this species inoculated. Recovery of the virus from *P. serotina* to a single tree of *P. glandulosa* was recorded, but the latter may have become naturally infected in the field plots rather than as a result of the experimental indexing from *P. serotina.*

PATHOGENESIS OF X-DISEASE VIRUS IN CHOKECHERRY. —*Recovery of the virus from dormant chokecherries.*—Early experiments indicated that the X-disease virus could be efficiently transmitted from dormant buds of infected chokecherry. An experiment to confirm this finding was begun March 17, 1950. Young chokecherries just breaking dormancy were inoculated by grafting scions obtained from 4 dormant diseased chokecherries in the field. A series of 6 plants was grafted with each source of inoculum. The single control series was inoculated from 2 greenhouse plants that were exhibiting symptoms. Transmission of the virus from the dormant diseased chokecherries was apparent on June 26, 1950. Failure to transmit the virus from symptom-expressing greenhouse sources was undoubtedly due to failure of the graft unions (Table 2).

TABLE 2.—*Transmission of X-disease virus from aerial parts of dormant diseased chokecherries by grafting. 1950*

Chokecherry source tree[a]	Number of trees grafted (March 17)	Number of living grafts (April 15)	Number of trees infected (June 26)
F-13A	6	2	2
F-13B	6	3	4
F-13C	6	2	2
F-13D	6	3	0
XM-1[b]	2	0	0
XM-5	4	0	0

[a] Cultures labeled "F-" are identified in Table 1.
[b] Sources XM-1 and XM-5 were expressing symptoms of X-disease when grafts were made.

Longevity of the virus in excised budsticks of dormant chokecherry.—Stoddard (6) reported that the virulence[6] of the X-disease virus progressively decreased in excised peach budsticks, declining rapidly after the second day and totally disappearing within a week after excision. Loss of virulence[6] occurred long before the buds because incapable of union.

Budwood of 4 dormant X-diseased chokecherries located in Livingston County, New York, was obtained on January 17, 1951, and stored at 34–36° F. for the duration of the experiment. Buds taken after 5 different storage intervals were inserted into 1-year-old Lovell peach seedlings. Transmissibility of the X-disease virus was not altered materially at the end of 35 days' storage (Table 3).

[2] *P. dunbari* Rehd. is a cross of *P. maritima* and *P. americana.* The seedlings employed in this study were segregates and undoubtedly did not possess the exact genotype of the clonal selection of *P. dunbari.* In appearance, they closely resembled *P. besseyi.*

[3] Material received under this name from commercial nurseries was probably *P. glandulosa.*

[4] Inoculating chokecherry buds grew and resultant foliage showed anthocyanosis.

[5] A cross of (*P. americana* × *P. salicina*) and *P. cerasifera pissardi.*

[6] "Virulence" as used by Stoddard (6) is undoubtedly equivalent to "transmissibility of the virus" in this instance.

TABLE 3.—*Transmission of the X-disease virus to peach from dormant diseased chokecherry budsticks after various intervals of storage, 1951*

Chokecherry source tree[a]	Number of trees inoculated	Trees infected from buds stored for indicated periods (days)[b] 1	5	12	21	35	Number of trees infected (June 3, 1951)
XD-1	10	1/2[c]	2/2	2/2	2/2	1/2	8
XD-2	10	2/2	2/2	1/2	1/2	1/2	7
XD-3	10	0/2	1/2	2/2	1/2	2/2	6
XD-4	10	2/2	2/2	1/2	2/2	2/2	9
HD-1[d]	10	0/2	0/2	0/2	0/2	0/2	0
Unbudded control	10	0/–	0/–	0/–	0/–	0/–	0

[a] See Table 1 for identity of cultures.
[b] Budsticks were obtained January 17, 1951, and stored at 34–36° F.
[c] Numerator of the fraction denotes number of trees infected; the denominator, the number inoculated.
[d] Tree did not exhibit X-disease in 1951 and was presumed noninfected.

Presence of the X-disease virus in chokecherry roots.—Eleven large woody root sections with attached fibrous roots were removed from X-diseased chokecherries and potted in a light sand-soil mixture. Eight root sections failed to produce any aerial growth, but 3 obtained from 2 different trees produced moderately vigorous aerial shoots. In all instances, this growth exhibited the anthocyanosis characteristic of X-disease 6–8 weeks after growth began.

Distribution of the X-disease virus in diseased chokecherry.—When large chokecherry trees in the field were inoculated with the X-disease virus by budding, symptoms of X-disease developed initially only in the inoculated branches. Anthocyanosis of the remainder of the trees developed relatively slowly, and considerable portions of some trees did not show symptoms of X-disease a year after inoculation. In a few cases, some shoots failed to show symptoms 23 months after inoculation.

In August, 1949, 11 months after inoculation, budwood was selected from symptomless branches 12–18 in. away from symptom-expressing limbs on 3 infected trees, and used to inoculate young chokecherry seedlings. Control inoculations were made with buds from anthocyanotic portions of the same trees. The virus was transmitted from buds taken from anthocyanotic branches of 2 of the 3 source trees, but none of the 16 inoculations made with buds from symptomless branches of the same trees resulted in transmission of the virus.

STUDIES OF THE EFFECT OF X-DISEASE IN COMBINATION WITH CERTAIN OTHER STONE FRUIT VIRUS DISEASES.—Presence of the necrotic ring spot virus in many of the X-diseased chokecherries that exhibited the proliferated symptom (Table 1) suggested that this symptom might be due to the combined effect of necrotic ring spot virus or, possibly, other viruses and the X-disease virus.

To determine the effect on the expression of X-disease of other viruses that might have been or were known to be in certain of the X-disease cultures as contaminants, combination inoculations were made on 3 peach varieties (Elberta, Halehaven, and Rochester), pin cherry (*P. pensylvanica*), and chokecherry. In August, 1948, 5 series composed of several trees of each of the hosts were inoculated with X-disease virus from infected chokecherries. Series I was, in addition, inoculated with buds of sour cherry infected with green ring mottle virus and a latent necrotic ring spot virus. Series II was, in addition, inoculated with buds of sour cherry infected with viruses of sour cherry yellows, prune dwarf, and a latent necrotic ring spot. Series III was, in addition, inoculated with buds of sour cherry infected with a latent necrotic ring spot virus. Series IV was, in addition, inoculated with buds of sour cherry infected with sour cherry yellows virus and a recurrent necrotic ring spot virus. Series V received no inoculations other than X-disease virus, and a sixth series was retained as noninoculated controls. All inoculated trees were 6–8 years of age and were growing in the field.

Shock symptoms attributable to infection with the necrotic ring spot virus were obtained in all 3 species in series I, II, III, and IV. Certain trees of series V also showed shock symptoms of necrotic ring spot. Symptoms were especially severe on the 3 peach varieties and consisted of delayed foliation, dieback, and severe leaf necrosis. No symptoms attributable to the green ring mottle, cherry yellows, or prune dwarf viruses were evident in any of the species in 1949 or 1950.

X-disease symptoms appeared in chokecherry, but not in any of the peach varieties or in pin cherry, although an indexing of inoculated pin cherries later revealed that the X-disease virus had been transmitted. Failure to transmit X-disease virus to peach was probably due to the presence of necrotic ring spot virus as a contaminant in many of the X-disease inoculations. The pronounced necrosis of peach tissue incited by the necrotic ring spot virus at the sites of bud insertion prevented union of buds and stocks.

No alteration in the symptoms of X-disease in the infected chokecherry trees was noted, irrespective of the additional virus or viruses that were incorporated in the inoculations. Data on chokecherry are tabulated in Table 4.

DISCUSSION.—Several different virus-host relationships in chokecherry as contrasted to the virus-host

TABLE 4.—*Infection of chokecherries with X-disease virus in combination with other viruses*, 1949-1950

Virus combination used	Trees inoculated (number)	Trees with X-disease (number)
Sour cherry green ring mottle, necrotic ring spot,[a] and X-disease	8	5
Sour cherry yellows, necrotic ring spot,[a] prune dwarf, and X-disease	8	5
Necrotic ring spot,[a] and X-disease	4	0[b]
Necrotic ring spot,[c] sour cherry yellows and X-disease	5	4
X-disease only[d]	14	9
All combinations containing necrotic ring spot and X-disease[e]	25	14

[a] A strain latent in Montmorency sour cherry after initial acute symptoms.

[b] Failure probably due to the low transmissibility of the strain of X-disease virus (F-43) employed.

[c] This strain usually incites striking annual symptoms in Montmorency sour cherry following initial acute symptoms.

[d] Certain of the cultures were later found to be contaminated with latent necrotic ring spot virus.

[e] Summation of all inoculations in which necrotic ring spot virus was intentionally used.

relationships in peach as reported by Stoddard (6) were found. Stoddard (6) failed to recover the virus from aerial parts of dormant diseased peaches or from growing diseased trees before foliage symptoms appeared. He found that transmissibility of the virus in peach budsticks declined rapidly after the second day of excision and had totally disappeared by the end of a week.

In Wisconsin, X-disease virus was recoverable from aerial parts of diseased chokecherries at any time during the growing season or during dormancy. In New York, the virus was also transmitted readily from dormant trees and persisted in excised budsticks of dormant diseased chokecherries for 35 days at 34–36° F.

Palmiter and Hildebrand (1) concluded that the virus was present in symptomless branches of partially diseased peaches, since pruning out infected limbs did not afford control of the disease. Stoddard (6), however, was unable to recover the virus from symptomless branches of diseased peaches. In the present study, the virus was, likewise, not recovered from symptomless branches of partially diseased chokecherries if such branches were located 12–18 in. from diseased branches. Movement of the virus from inoculation sites proceeded relatively slowly in large chokecherry trees.

Delay in expression of foliage symptoms following commencement of growth in diseased trees was noted in peach by Stoddard (6) and Palmiter and Hildebrand (1). A similar delay was observed in chokecherry in the present study. Stoddard (6), attempting to explain this delay in peach, postulated that the virus was either inactivated or entirely translocated away from the aerial parts of diseased peach with the advent of dormancy, and that the aerial parts were reinfected from the roots each year after growth commenced. Neither of these hypotheses affords an explanation of the delay in symptom expression in chokecherry following commencement of growth since the virus was as readily transmitted from dormant tissues as from tissues during the growing period.

Host range studies in the genus Prunus revealed possible immunity to the X-disease virus in only 1 species (*P. serotina*) of the 22 species tested. Several hosts were, however, symptomless carriers of the virus. Short-term field studies of large trees of 1 symptomless carrier (*P. pensylvanica*) did not reveal obvious deleterious effects of infection with X-disease. It is probable that this host, and others that react in a similar manner, are potentially more serious reservoirs of the X-disease virus than chokecherry, in which the disease tends to be self-eliminating by causing death of the host.

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MULTIPLICATION OF PSEUDOMONAS TABACI IN LEAVES OF BURLEY TOBACCO, NICOTIANA LONGIFLORA, AND HYBRIDS[1]

Stephen Diachun and Joseph Troutman

SUMMARY

Leaves of susceptible (*Nicotiana tabacum* L. var. Ky 56), resistant (*N. longiflora* Cav.), and hybrid (TL106 and 1819-9) plants were inoculated with *Pseudomonas tabaci* (Wolf & Foster) St. by water soaking. The bacterial population in the inoculated areas was determined by plating daily for a period of about a week. The bacteria were found to multiply inside inoculated leaves of all 4 kinds of plants. Multiplication was greatest in the susceptible, least in the resistant, and intermediate in the hybrid plants.

All commercial varieties of burley tobacco now in use are susceptible to wildfire, a bacterial leaf spot disease caused by *Pseudomonas tabaci* (Wolf & Foster) Stevens. Wildfire has been very destructive in Kentucky in recent years. Most plants of *Nicotiana longiflora* Cav. are resistant to or immune from wildfire. Burley tobaccos resistant to wildfire are being developed from crosses or hybrids between tobacco (*N. tabacum* L.) and *N. longiflora* (3, 4, 6).

Evidence is accumulating to show that certain fungi and bacteria pathogenic on some plants may grow or multiply to some extent in or on tissues of other plants without causing symptoms (1,2,8). For example, *Xanthomonas phaseoli* (E. F. Sm.) Dowson, the cause of common halo blight of beans, can multiply to some extent in soybeans without producing symptoms; conversely *Pseudomonas glycinea* (Coerper) Stapp, the cause of bacterial blight of soybean, can multiply in beans without producing symptoms (1).

The purpose of this study was to determine whether *P. tabaci* would multiply in leaves of selected resistant plants of *N. longiflora* and, if so, to compare the rate of multiplication in leaves of resistant (*N. longiflora*), susceptible (Ky 56), and hybrid plants. An abstract of part of this work has been published (5).

PROCEDURE.—Tests were made with 24-hour beef-peptone broth cultures diluted 1:200, 1:1000, and 1:10,000 with sterile distilled water. Inoculations were made into 3 greenhouse plants of *N. longiflora*, Clayton's wildfire-resistant TL 106 (3), Ky 56, a wildfire-susceptible burley, and strain 1819-9, a wildfire-resistant burley. One leaf of each plant was inoculated by water soaking. A strong stream of inoculum was forced from a hypodermic syringe and directed against the lower leaf surface when stomata were known to be open. As the liquid was forced into the leaf through the stomata, that part of the leaf in which the air in the intercellular spaces was replaced by the liquid became visibly water-soaked. By moving the syringe (and the stream of inoculum) as the contents were forced out against the leaf and by refilling the syringe several times, a continuous strip about 2 cm. wide consisting of several adjoining stripes was water-soaked on both sides of and adjacent to the midrib. After the evidence of water soaking disappeared (20–30 minutes), the leaves were rinsed with sterile water. The population of bacteria in the inoculated area of each leaf was determined daily, the first sample being taken less than an hour after inoculation. Samples were taken on 7 consecutive days after inoculation and then on 1 or 2 later dates. A disc 5.5 mm. in diameter was cut from a leaf with a sterile cork borer, placed in a drop of sterile water in a sterile test tube, and crushed thoroughly with a sterile rod. One ml. of sterile water was added, stirred thoroughly, and allowed to stand at least 15 minutes. Dilution plates were then poured with potato dextrose agar. After 48 or 72 hours, colony counts were made.

RESULTS AND CONCLUSIONS.—As shown in Table 1, *P. tabaci* multiplied inside leaves of all 4 kinds of plants. Multiplication was greatest in Ky 56, least in *N. longiflora*, and intermediate in the hybrids TL 106 and 1819-9. Inoculated areas of Ky 56 were slightly yellow 2 days after inoculation; in 3 days the inoculated tissue appeared scalded and collapsed; in 4 days it was thin and drying out. The bacterial population seemed to reach a peak at about the time the tissue was killed; later the number of bacteria recovered was reduced or variable.

No symptoms developed on *N. longiflora* and TL 106. On 1819-9 very small necrotic specks, reminiscent of very mild angular leaf spot, began to appear 4 days after inoculation. They did not increase in size

TABLE 1.—*Multiplication of Pseudomonas tabaci in leaf tissue of susceptible (Ky 56), resistant (N. longiflora), and intermediate (TL 106 and 1819-9) plants inoculated with a culture diluted 1:1000*

Days after inoculation	Mean number of viable bacteria recovered from			
	Ky 56	N. longiflora	TL 106	1819-9
0	40	45	45	20
1	180	50	110	205
2	40,000	250	560	3,600
3	200,000	250	1,500	3,300
4	150,000	250	1,700	1,400
5	117,000	90	3,400	400
6	102,000	35	550	1,600
7	100,000	160	140	960
14	23,000	0	240	1,300

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TABLE 2.—*Multiplication of Pseudomonas tabaci in inoculated leaves of susceptible (Ky 56) and resistant (N. longiflora) plants*

Days after inoculation	Mean number of viable bacteria recovered from Ky 56 Inoculated with culture diluted			*N. longiflora* Inoculated with culture diluted		
	1:200[a]	1:1,000	1:10,000	1:200[a]	1:1,000	1:10,000
0	300	32	12	250	18	6
1	15,300	16,000	430	2,100	2,000	420
2	5,600,000	2,400,000	275,000	10,000	23,000	2,400
3	2,600,000	1,700,000	1,000,000	4,000	2,700	1,600
4	21,700	1,280,000	2,000,000	2,300	6,600	1,600
5	1,200,000	117,000	360,000	7,000	18	210
6	170,000	3,450	82,000	6,300	300	10
7	–	68,700	1,200	–	500	10
10	–	17	6,000	–	1	7
17	–	265,000	238,000	–	5,000	800

[a] Test with 1:200 dilution was not concurrent with the other series.

but remained very small throughout the period of observation.

In *N. longiflora* and in the hybrids TL 106 and 1819-9 the bacterial population seemed to reach a peak at about the same time as in burley; then the number of viable bacteria decreased or fluctuated. The reason for the decrease in number is not known. In culture, with a decrease in the pH of the medium, these bacteria die in a few days (7). Possibly a similar condition may develop inside the inoculated leaf tissue.

When leaves were inoculated with more concentrated cultures, the increase in the population was perhaps somewhat more rapid, but the general pattern of increase and then erratic decline was essentially the same (Table 2).

The reason for the fluctuation in the number of bacteria recovered after the peak is reached is unknown.

Inasmuch as *P. tabaci* can multiply in leaf tissue of resistant plants, the possibility exists that, in time, strains of the organism that could cause symptoms in plants now resistant might evolve.

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SEEDLING AND MATURE PLANT INOCULATION OF SUDAN GRASS WITH HELMINTHOSPORIUM TURCICUM [1]

P. N. Drolsom and J. G. Dickson

SUMMARY

Seedling plants of 321 lines and 5 check varieties of Sudan grass were grown in the greenhouse in 1949-50 and inoculated with the leaf blight organism, *H. turcicum*. Disease readings were taken on leaf flecking and leaf blighting. Leaf blight appeared to be the important phase of the disease. The seedling reaction to disease in the greenhouse did not give a classification for resistant and susceptible types that agreed satisfactorily with mature plant reactions obtained in the field.

Environment was an important factor in leaf blight development in Sudan grass. Rainfall and temperature influenced the time of appearance of the disease in epiphytotic proportions.

The date of planting in relation to severity of leaf blight development in Sudan grass was of little significance. There was some indication in 1950 that later seedings were more heavily diseased but subsequent data did not confirm this. In the field leaf blight appears often to be a disease of later growth stages, but this may be closely associated with inoculum build-up.

In the 1 year in which the method was used, diseased leaves spread about the nursery resulted in good infection and disease spread. Spreader plants provided excellent sources of inoculum and were effective in disease spread throughout the season. Spray inoculation with conidia gave variable results and was the least desirable of the infection methods tried.

Leaf blight, incited by *Helminthosporium turcicum* Pass., is one of the serious foliage diseases occurring on Sudan grass in the middle west. *H. turcicum* also incites corn leaf blight which has become increasingly important in recent years. The present study concerns the reaction of Sudan grass seedlings to *H. turcicum* and the production of field epiphytotics of the disease.

Apparently, *H. turcicum* was first reported on Sudan grass grown in the United States in 1936 (1). The morphology of this organism was described by Drechsler (3), and physiological studies were reported by Mitra (4), Nisikado (5), and Nisikado and Miyake (6). Variability in *H. turcicum* has been investigated by Robert (7) and Robert and Jenkins (9). Robert (7) tested hyphal-tip isolates from a single conidium and found that they differed in pathogenicity and cultural behavior.

In breeding, investigators are continually searching for better methods of testing for disease reaction in the selection program. Seedling inoculation studies to aid in selecting resistant types are useful with a number of economic plant species. Early, effective elimination of disease susceptible individuals was the principal objective in the seedling study on this disease.

Artificial epiphytotics are necessary each season to permit selection for resistant types; therefore, a portion of this investigation was concerned with finding methods of inoculation suitable for use in the field. Conventional methods of preparation of inoculum become inconvenient when large quantities are needed for field plots. Robert and Findley (8) reported the use of dried leaves, stored from the previous season and spread throughout the nursery the following summer, to produce the disease. Ullstrup (11) used this method for inoculation of corn with the Southern leaf blight organism, but found it unreliable, especially in dry seasons. Previous work at the Wisconsin station has indicated that hypodermic inoculation gave inconsistent infection, and the use of a spore suspension spray in humid evenings and nights was not entirely satisfactory.

MATERIALS AND METHODS.—*Greenhouse tests.*—In the seedling inoculation experiments, 30–40 seeds of each lot tested were planted in a soil-sand mixture in 2-in. pots. Three replicate series were grown at different dates. Greenhouse temperatures were maintained at or near 26° C. for the period of the experiment. Seedlings were inoculated when the majority of plants were in the third or fourth leaf stage. A culture of *H. turcicum* isolated from Sudan grass grown at Madison, Wisconsin, was used. The fungus was grown on potato-cerelose agar in 150 ml. bottles for a minimum of 18 days. Inoculum was prepared by scraping conidia and mycelium from the agar slants and mixing in a Waring Blendor. This suspension of mycelium and spores was sprayed on the seedlings. The plants were then placed in a closed chamber for 36 hours with humidity near saturation at 26° C.

Two types of disease readings were made, leaf flecking and leaf blighting. An estimate of leaf flecking was taken 2 days after removal of the plants from the moist chamber. Flecks were all about the same size, therefore, classes 1–4 were established on the basis of average number per leaf blade, 4 indicating over 70 per cent of the area covered by pin-point, dark flecks. An estimate of leaf blighting was taken 7–10 days after seedlings were removed from the moist chamber. Lesion spread from some of the original flecks was characteristic and classes 1–4 were used on the basis

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of both number and area of leaves blighted, 4 indicating complete blighting of all leaves inoculated.

The varieties used and their reactions to *H. turcicum* were: Tift, resistant to moderately resistant; Piper, resistant to moderately resistant; Sweet Sudan, moderately susceptible; Commercial, susceptible; and (Wis. sel.) Line 109, very susceptible.

Additional greenhouse tests were run to determine the effect of temperature on disease incidence and development. Plants were inoculated and grown at controlled temperatures of 16°, 20°, 24°, and 28° C. Methods of inoculation and disease readings were the same as in other experiments.

Field tests.—Small plots of the control varieties and Rox Orange Sorghum (highly resistant) were seeded in isolated locations in the summers of 1950, 1951, and 1952. These plots were used to investigate various methods of obtaining field epiphytotics and to determine if age of plants at various periods of the season affected severity of disease development. Four seedings were made at 10-day intervals, beginning about June 1. These were arranged in a split-plot design with 3 replicates and consisted of 5- or 8-ft. rows for each strain.

Each isolated plot was given a different inoculation treatment, with 1 plot remaining untreated. The inoculation methods used in 1950 and 1951 were: 1) spray inoculation, 2) hypodermic inoculation, and 3) transplanting diseased Sudan grass plants from the greenhouse at the ends of the rows in the plot. *H. turcicum* was grown on oat hulls to obtain conidia for the 1950 spray inoculation trials and conidia from leaves were used in subsequent experiments. In 1951 and 1952 all inoculum was obtained from diseased Sudan grass leaves stored over winter. Prior to inoculation the leaves were placed in a moist chamber 48 hours and the conidia washed off with water. Inoculum for the 1950 hypodermic inoculations was grown on potato-cerelose agar. Inoculations were made with a syringe, placing the inoculum in the leaf primordia about 8 in. below the leaf whorls of plants 2–3 ft. high. Inoculated plants were considered diseased when blight developed around the needle punctures in the leaves. The spreader-plant method of producing a field epiphytotic necessitated starting the plants in the greenhouse. Line 109, a selection of Sudan grass very susceptible to leaf blight, was used. When 24–36 in. in height, the plants were inoculated with a water suspension of conidia and kept in a moist chamber for 36 hours. They were then transplanted in the alleys at the head of alternate rows and in the border rows.

Inoculation with plant material was tried in an isolated location in 1952. Diseased leaves were collected in 1951 and stored in a dry place at room temperature. Fifty leaves were scattered at random within and between rows on July 24 and 50 leaves on August 8, in an isolation plot 30 × 48 ft. in area.

The disease rating scale used in classifying plants for reaction to *H. turcicum* in the field comprised 10 classes from "1" (resistant) to "10" (very susceptible). Number and size of leaf lesions were the principal criteria which determined disease ratings. In all isolation plantings, disease readings were made on a row basis. The class with the largest number of plants determined the row classification.

TABLE 1.—*Numbers of lines of Sudan grass seedlings in the respective classes for blighting and flecking, indicating relationships among the 3 series and 4 classes, with chi-square values*

Class	Blighting Series 1	2	3	Totals	Flecking Series 1	2	3	Totals
1	151	118	90	359	206	143	118	467
2	125	126	155	406	114	128	171	413
3, 4	75	107	106	288	31	80	62	173
Totals	351	351	351	1053	351	351	351	1053
		$X^2 = 26.741^{**}$				$X^2 = 60.345^{**}$		

** p exceeds 0.01

RESULTS. — *Greenhouse.* — In the 1949-50 seedling test, 321 lines and 5 check varieties were grown and inoculated in 3 different series in the greenhouse and disease data obtained. The chi-square test of independence was used to determine the association among the 4 classes and the 3 series for flecking and blighting (Table 1).

The chi-square values for both categories of disease were highly significant, indicating an association between disease class and series. The test was only approximate as it did not take into account the shift in individual lines between classes from one series to another.

A classification given in one series was not always consistent with the classification given the same line in another series. Such shifting of flecking and blighting classes within the 3 series made the results from seedling tests of questionable value.

Significant associations were obtained between blighting and flecking for each series. Since the shift in classes from one series to another was fairly extensive, however, the importance of the associations between blighting and flecking would tend to be minimized. Apparently flecking alone was not damaging to the Sudan grass seedlings, and since some lines of seedling plants displayed flecking without blighting, the data for flecking were of slight importance.

Two hundred ninety-six lines tested in the greenhouse were seeded in the breeding nursery in 15-ft. rows during the summer of 1950. Each row was graded according to relative severity of leaf blight on the basis of "1" being highly resistant and "6" very susceptible. The ratings were compared with the most severe fleck and blight notes obtained from the seedlings of the lines in the greenhouse tests. The chi-square test of independence was used to study the relationship. Results are in Table 2.

TABLE 2.—*Number of lines of Sudan grass showing association between seedling reaction in the greenhouse and mature plant reaction in the field, with chi-square values*

Field Blighting Class	Greenhouse Flecking Class 1	2	3, 4	Totals	Greenhouse Blighting Class 1	2	3, 4	Totals
1, 2	21	29	18	68	14	37	17	68
3	15	36	35	86	10	34	42	86
4	13	28	33	74	11	24	39	74
5, 6	13	29	26	68	1	23	44	68
Totals	62	122	112	296	36	118	142	296
			$X^2 = 8.343$				$X^2 = 28.265$**	

** p exceeds 0.01

Combining of classes was necessary because of small numbers in the extreme groups. There was no association between greenhouse flecking and field blighting. However, association between greenhouse blighting and field blighting was highly significant. Even though statistical significance was indicated, it was concluded that the association was not close enough to be of value in a breeding program. To be reliable, a greenhouse procedure must give a seedling test whereby few lines would be escapes, and this was not the case in the trial reported.

Field tests.—Data from the field isolation plots were subjected to analysis of variance. The locations serving as check plots were not included in the analysis because no leaf blight was observed in the control plots in 1950 and 1951 and only a small amount in 1952. However, this did not eliminate the possibility of natural infection causing some disease in the other plots. The sorghum variety, Rox Orange, was not included in the analysis as it was resistant to blight in all the experiments. F values for varieties, treatments, and planting dates were generally significant. It was expected that varieties would be different, since susceptible and resistant types were used. In 1950 and 1951 the 3 treatments—spray inoculation, hypodermic inoculation, and spreader plants—were not equally effective in producing artificial epiphytotics of leaf blight. In 1950 the plot with spreader plants was most severely diseased, whereas hypodermic inoculation gave the heaviest infection in 1951. Two methods of inoculation, namely, distributing dry, diseased Sudan grass leaves and hypodermic inoculation, were tested in 1952. Severe infection was obtained with both treatments. The third and fourth date plantings appeared to be more diseased than those of the earlier dates of seeding during the 1950 and 1951 seasons, but the difference was less in 1951. The interaction of varieties × treatments was significant in 1952, indicating that varieties did not respond similarly to the 2 treatments. The behavior of the variety, Tift, may have produced this interaction. Tift appeared to have less disease resulting from hypodermic inoculation, whereas all other varieties had slightly more disease with this treatment, when compared with inoculation by the use of diseased leaves. The interaction between treatments × seeding dates was highly significant in 1950 and 1951, indicating that seeding dates did not give a similar response in disease development at the different locations.

TABLE 3.—*Results of chi-square test of relations between disease development and date of planting, in isolation plots testing methods for producing epiphytotics of leaf blight*

Relation between disease development and date of planting using	Chi-square[a]	P Value
Hypodermic inoculation		
1950	5.566	.20-.30
1951	2.863	.50-.70
1952	1.014	.90-.95
Spreader plants		
1950	11.978	.01-.02
1951	4.293	.30-.50
Spray inoculation		
1950	15.851	<.01
1951	1.500	.80-.90
Diseased leaves		
1952	2.546	.50-.70

[a] N = 60 row observations.

The data were analyzed also with the chi-square test of independence to determine if there was a relationship between disease reaction and date of planting. Although leaf blight reaction was graded on a basis of 10 classes, this scale could not be used for the contingency tables because of small numbers in the extreme classes. Therefore, the 10 classes were combined into 3 groups, and the third and fourth planting date were combined similarly, resulting in 3 × 3 contingency tables. Rox Orange sorghum was omitted since it was consistently resistant. Chi-square results are in Table 3.

The associations obtained in 1950 for severity of disease and date of planting were variable. Two inoculation methods indicated a high association, with the later planting dates developing more disease. Results did not appear conclusive. In 1951 and 1952 there was no association between blight development and dates of planting.

Results of hypodermic inoculations are in Table 4. A high percentage of infection was obtained in both seasons with the susceptible varieties, Commercial, Sweet Sudan, and Line 109. In 1952, Piper and Tift, moderately resistant varieties, gave a high percentage of infection when compared with the 1951 results.

DISCUSSION.—The chi-square test of data obtained from seedlings grown and inoculated in the greenhouse indicated an association between the 4 disease classes and the 3 series. This was only an approximate test and gave no clues as to its weaknesses. The variability in disease reaction from series to series makes the reliability of the greenhouse seedling test made under these particular conditions doubtful. The seedling test would be valuable in screening for disease resistant material if consistent results were obtained.

TABLE 4.—*Disease development in 1951 and 1952, resulting from hypodermic inoculation of field-grown Sudan grass plants with a spore suspension of Helminthosporium turcicum*

Variety or Selection	1951 Inoculated No.	1951 Infected No.	1951 Infected Per cent	1952 Inoculated No.	1952 Infected No.	1952 Infected Per cent
Sweet Sudan	49	37	75.5	27	15	55.6
Commercial	76	48	63.2	39	28	71.8
Tift	46	12	26.1	20	12	60.0
Piper	54	29	53.7	45	38	84.4
Line 109	235	187	79.6	64	56	87.5
Rox Orange	27	7	25.9	20	8	40.0
Totals	487	320	65.7	215	157	73.0

The variability in data from the seedling test may be due in part to improper conditions for infection. Environmental conditions for the host and pathogen may not have been at the optimum level. Temperature was an important factor in leaf blight development. Leaves were blighted more quickly following inoculation at 24° and 28° than at 20° C. Additional investigations are necessary to determine the most favorable environmental conditions for the seedling test.

Difficulties were met also in obtaining virulent cultures in the 1950-51 and 1951-52 greenhouse studies. Apparently the extreme variability in this pathogen was involved. Instability in the genus *Helminthosporium* is well known, especially from the work of Christensen and Davies (2) with *H. sativum*, Shands and Dickson (10) with *H. gramineum*, Robert (7), and Robert and Jenkins (9) with *H. turcicum*. In the present study, sectoring was observed on several occasions in monoconidial isolates.

Variability in the pathogen and inoculation effectiveness appeared to be the major factors responsible for the variable results obtained. In addition, the lines of Sudan grass that were tested were not completely homozygous, adding further to sampling error.

The importance of environmental conditions in relation to the occurrence of field epiphytotics of leaf blight was well illustrated. The relatively slow development of leaf blight in Sudan grass in the 1950 growing season was in marked contrast to disease development in the other 2 seasons. Heavy rains undoubtedly washed off spores, and cool nights probably reduced disease development. Greenhouse studies indicated that inoculation resulted in no blighting at 16° C. compared with severe leaf blight at 20–22° C. Therefore, periods with relatively warm nights and high humidity should be most favorable for disease development.

Field data indicated that natural epiphytotics occurred when inoculum was present and when environmental conditions were very favorable for disease development. As a breeder acquires more resistant lines, susceptible plants might easily escape detection due to insufficient inoculum unless artificial inoculation is used.

Under the conditions in these experiments, field development of disease was rather probable with or without inoculation. Spray inoculation resulted in the greatest variation in amount of disease. The use of spreader plants transplanted into the field from the greenhouse required more labor than the other methods but was relatively reliable in producing a high incidence of disease. The use of diseased leaves preserved from the previous season was the simplest procedure, and the results from 1 trial appeared to be satisfactory.

The 1950 data indicated that Sudan grass planted in early July was damaged more by leaf blight than that planted in early June. However, the data obtained in 1951 did not verify this conclusion, and the results in 1952 indicated little relationship between date of planting and disease severity. Furthermore, when leaf blight was severe, as in 1951 and 1952, plants apparently resistant earlier in the season appeared to become more susceptible as plant development progressed. It is not known whether this was due to host changes, pathogen changes, environmental conditions, or a complex of these factors. An increasing amount of inoculum, as the season progressed, may have contributed to the apparent susceptibility of resistant lines.

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RELATIVE STABILITY OF FIRE BLIGHT BACTERIA [1]

E. M. Hildebrand [2]

SUMMARY

The first blight organism, *Erwinia amylovora*, has been characterized as a relatively unstable plant beterium. The objective in this study was to discover the effects on variability in pathogenicity or virulence of continued bi-weekly saprophytic culture on artificial media for 5 years. This was an attempt to discover what occurs in a stabilized environment.

The cultures were assembled from around the world (Canada, 7; New Zealand, 15; U. S. A., 40) and constituted the most extensive and complete collection of this organism ever made. The cultures were isolated from pear, apple, quince, Crataegus spp., *Raphiolepis umbellata, Pyracantha gibbsii*, spirea, flowering almond, loquat, and medlar. Cultural purification involved isolation of 5 to 20 single cells from each of the 62 mother cultures, isolated originally by the poured-plate technique, making a total of 720 cultures. Based on pathogenicity and cultural identity the number of cultures was reduced to 136 before the end of the first year. These 136 isolates were employed in numerous morphological, physiological, and pathogenetical experiments. Green Kieffer pears collected about August 1 and stored in oiled paper at 32°C. were available for pathogenicity tests any time during the year.

Aseptic slices were regularly employed for evaluating virulence at 3-month intervals. The effect on virulence of successive culture transfers demonstrated more variability than stability during 5-years time. Of the 49 parents and 87 progenies, the daughter (single-cell) cultures maintained high virulence most consistently. Of the 42 cultures high in virulence in 1935, 17 had been high, 23 moderate, and 2 low in 1934. Of the 33 cultures nonvirulent or attenuated in 1935, 5 had been high, 18 medium, 4 low and 6 nonvirulent in 1934.

Length of the highly virulent bacterial cultures was significantly less than that of those low in virulence. Frequency of cells possessing flagella was related to virulence because more than 80 per cent of the cells of nonviruent cultures were without flagella. The number of flagella per cell, while variable, was always greatest for cultures high in virulence. The highest average number of flagella per cell (6.6) was had by Antario 3b, a highly virulent single-cell culture. The lowest number (1.0) was true for New Zealand 1, an attenuated parent culture. The length of flagella seemed to bear some relation to virulence because as virulence decreased the proportion of cells with short flagella increased.

Virulence showed practically no correlation with physiological behavior. Decline or loss of pathogenicity was never correlated with carbon and nitrogen utilization, but there was much variability in extent of activity. All 136 cultures were run through the tests of Determinative Bacteriology. None produced indol. None reduced nitrate to nitrite or nitrite to ammonia, but all produced ammonia. All fermented litmus milk and all liquefied gelatin, but with nonvirulent cultures producing as much or more liquefaction than those of high virulence.

In studies on pathogenicity, while there was much variability, the progeny (single-cell) cultures were somewhat more stable than the parent (single colony) cultures. It is believed that the more stable parent cultures were in truth the product of single cells.

Growth rates seemed to be related to virulence because loss of pathogencity was correlated with a shortening of generation time.

Fire blight is the oldest known bacterial disease of plants. It was well known for a century before its true cause was discovered in Illinois by Burrill (3). Fire blight is most severe in the warm southern states where it is practically impossible to grow high quality pears. Inherently fire blight bacteria (*Erwinia amylovora*) multiply at relatively rapid rates when proper conditions are provided, with new generations appearing within approximately 1 hour (8).

Most bacteria are remarkably stable and Brierley

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(2) pointedly states . . . "this implies an extremely delicate and constant hereditary mechanism, quite comparable in its behavioristic fixety and nicety to that in higher animals." It is significant that most plant bacteria are more stable under favorable growing conditions than under conditions such as those preventing rapid vegetative growth, and that much of the variation is environmental rather than genetic in character.

The fire blight organism, unlike the crown gall organism, is relatively unstable and variable in culture and in nature. The cause for its sporadic character and the fluctuations in the severity of its attacks have been sought in the pathogen (11), but the factors of the environment and the growth status of the susceptible host now appear to share much of the responsibility for the severe outbreaks (6, 7). It is agreed that disease-resistant plants constitute a long range ideal for which to strive in fire blight control, but efforts for plant improvement through breeding will be futile unless the resistance achieved is applicable to all strains of the organism. Therefore, the objective is to determine the status of the causal organism of fire blight in relation to its stability and activity in culture media separated from plants. Data presented herein have been culled from an extensive study of variation of 136 isolates of this organism. The study is concerned with variation of isolates on culture media over a 5-year period (1933-1937).

THE ASSEMBLY OF REPRESENTATIVE CULTURES.—In 1932 bacterial cultures were obtained from nearly every place of importance where fire blight was known to occur in the world, from most fruit growing states in the United States and from Canada and New Zealand. Disease specimens simulating fire blight were also obtained from Asia and Europe, but none of these yielded the fire blight organism. A total of 62 original cultures were assembled—7 from Canada, 15 from New Zealand, and 40 from the United States. They were isolated from 10 different suscepts including pear, apple, quince, Crataegus spp., *Raphiolepis umbellata*, *Pyracantha gibbsii*, spirea, flowering almond, loquat, and medlar. In addition, 10 cultures were isolated from the most limited range possible or from the blossoms in 10 different locations in a large Duchess apple tree in Ithaca, N. Y. The parent and single-cell cultures found to be identical to other isolates from the same source, in cultural and pathogenicity studies during the assembly period, were reduced to single representatives, keeping in the collection only 1 representative for each cultural difference. Thus the original cultural selection was made for differences rather than identity of behavior. All isolates which failed to produce moderate infection on 3 successive inoculation trials were discarded.

PURIFICATION OF CULTURES BY SINGLE CELL ISOLATION.—Decline in virulence, loss of pathogenicity, and other variations were regularly encountered when single colony cultures of the fire blight organism were grown in artificial culture media. This led directly to making 5–20 single cell isolations from each of the 62 parent cultures covering a period of about 8 months. Thus a total of approximately 720 cultures were assembled.

Comparative studies on similarities and differences based on cultural, morphological, and pathogenic grounds demonstrated close similarities between parent cultures and their single cell progenies. After the first comparative study, in which duplicate, closely similar cultures were eliminated, the total number of cultures was reduced from 720 to 226. After the second elimination, involving 3 successive pathogenicity tests, the total was reduced to 136 at about the close of the first year of study and included 49 parent and 87 progenies. By this time it was believed that all contaminated cultures had been discarded.

Variability in cultures, it was soon learned, was greatly reduced and stabilized by employing plain nutrient broth minus sugar as the stock culture medium. The biweekly transfers were made in duplicate to both nutrient agar and nutrient broth from nutrient broth. Cultural variability was most conspicuous on nutrient agar from which, at 1 or 2 days of age, the inoculum was taken for the various morphological and physiological studies.

The numerous comparative studies, employing 136 cultures, made during this investigation on morphology, physiology, and pathogenicity led to the publication of a preliminary report (11) where it was stated that no strain was believed to be of significance in any breeding program for disease resistance. Other unreported studies confirm the work of Ark (1) that strains differing distinctly in pathogenicity may exist in nature.

PATHOGENICITY.—*Fire blight ooze and toxin production.*—Suitable host materials (blossoms, shoots, fruits) for testing pathogenicity are rather restricted and of short duration; hence the desirability of obtaining something better. The criterion for pathogenicity or virulence is toxic plasmolysis of cells in susceptible tissues with resultant discoloration and death plus bacterial ooze production. Fire blight ooze can be induced readily only when host tissue is being attacked by the pathogen. By employing aseptic slices of green Kieffer pear fruits stored at 32°C., it was possible to produce sizable quantities of ooze at will. This ooze was demonstrated to contain a thermostabile toxic substance (10). This toxic substance has never been produced in artificial or synthetic culture media. The fire blight organism perishes in a few days' time in its own liquid ooze but will survive for months or even years (for over 3 years in these studies) if the milky ooze is promptly dried upon exudation from the dead or dying plant tissues. Aseptic slices from green Kieffer pears collected about August 1 and stored at 32°C. proved ideal for pathogenicity tests anytime during the year.

Effect on virulence of successive culture transfers.—After preliminary trials the frequency of transfer of stock cultures was set at 2 weeks. Table 1 summarizes the effect on virulence of successive bi-weekly trans-

TABLE 1.—*Effect on virulence of successive bi-weekly transfers of the fireblight organism in nutrient broth during a 5-year period. (See text)*

Virulence[a]	1933	1934	1935	1936	1937	1933-37[b]
High (H)		38	H 17 M 32 42 L 2 N 0	H 18 M 10 33 L 2 N 3	31	8, 15, 8
Moderate (M)	136	80	H 13 M 24 L 1 39 N 1 H 3	H 12 M 8 L 3 26 N 3 H 8	24	6, 18, 0
Low (L)		9	M 15 22 L 2 N 2	M 12 39 L 12 N 7	38	0, 2, 36
Non-virulent (N)[c]	0	9	H 5 M 18 33 L 4 N 6	H 4 M 9 38 L 5 N 20	43	2, 2, 39

[a] Based on 2 reactions produced by inoculation on slices of green Kieffer pear fruits at the end of 7 days.

[b] The 3 numbers indicate, respectively, cultures consistently at a given level of virulence, cultures making 1 departure from that level, and cultures making more than 1 departure.

[c] Note the gradual increase of nonvirulent cultures through the years. This also appears to apply to cultures low in virulence. Note that the moderately virulent cultures show the biggest drop in numbers.

fers of the fire blight organism in nutrient broth during a 5-year period. In 1933, the first year, all 136 cultures were pathogenic and moderate or high in virulence, based on tests employing succulent Bartlett and Clapp Favorite pear shoots and slices of green pear fruits as susceptible host material. By 1934, 9 cultures had become attenuated or nonvirulent and 9 low in virulence. From 1934 on, all suscept reactions were tested quarterly in duplicate on green Kieffer pear fruits and recorded at the end of 7 days. Detailed examination of the table shows that the fluctuations in virulence were considerable from year to year. Also, a small percentage of cultures losing virulence 1 year had regained virulence after attenuation within a year's time, or after approximately 26 bi-weekly transfers of the organism. Virulence tests were made at 3-month intervals and the changes were not so abrupt as the table giving the results at the year's end might indicate.

Of particular significance was the fact that the nonvirulent or attenuated cultures usually remained smooth (S), a condition comparable to the attenuated cultures Ark (1) reported after passage through 10 per cent sucrose broth. The effect on virulence of successive bi-weekly transfers to nutrient broth demonstrated more variability than stability during 5 year's time.

MORPHOLOGY.—*Virulence in relation to length of bacteria.*—The cells of highly virulent bacterial cultures were significantly shorter than those of medium or low virulence and of nonvirulent or attenuated (Table 2). Results on relation of length of cells to pathogenicity confirm and extend those of Ark (1). The Congo Red negative stain was employed. Cells of the most virulent individual culture were less than 1μ long (0.87μ). However, 2 of them equalled the mean average size. Width of cells was much less variable and relatively nonsignificant statistically except for the 3 nonvirulent mucoid (M) cultures, which measured nearly twice the length of the cultures high in virulence and had a significantly greater width.

TABLE 2.—*Length of bacteria in relation to virulence*

Degree of virulence	Number of cultures	Mean size (microns)
High	9	1.42 × .67[a]
Medium	12	1.57 × .71
Low	18	1.61 × .72
Nonvirulent	18	1.59 × .68
Average	57	1.56 × .69
Average (mucoid)	3	2.75 × .87

[a] The length of the highly virulent bacterial cultures was significantly less than that of those low in virulence (odds >20:1).

Frequency of cells possessing flagella.—When microscopic examination was made of a large number of cultures in succession in hanging drops, there seemed to be differences in motility between cultures, especially as regards the proportion of cells swimming and their speed of movement. This led to the staining of all the cultures employing the Casares-Gil and Gray's flagella stains. When studied for frequency of cells possessing flagella the only case considered significant was for the 10 nonvirulent cultures in which less than 20 per cent of the cells possessed flagella. Table 3 gives the results when a random selection of 106 of the cultures was classified according to degree of virulence, based on the pathogenic reaction produced at the end of 7 days on cut slices of green pears. Loss of virulence seemed to be related to loss of flagella.

Number of flagella per cell in relation to virulence.—The number of flagella per cell, while variable even within individual cultures, was always greatest for the

TABLE 3.—*Frequency of cells possessing flagella in 106 cultures of the fire blight organism selected at random from the collection of 136 cultures*

Degrees of virulence	Number of cultures	Frequency of cells possessing flagella[a]		
		High	Moderate	Low
High	28	11	7	10
Medium	39	9	16	14
Low	29	8	5	16
Nonvirulent[b]	10	0	0	10

[a] When over 60 per cent of the cells in 3 microscopic fields had flagella the frequency was rated high; when 20 to 59 per cent, moderate; and when less than 20 per cent, low.

[b] Attenuated or nonvirulent cultures which at one time had been virulent showed more than 80 per cent of cells without flagella.

TABLE 4.—*The number and length of flagella per cell in relation to the degree of virulence of 106 cultures of the fire blight organism*

Degree of virulence	Number of cultures	Average number of flagella per cell	Average length of flagella per cell
High (H)	28	3.35	5.6
Medium (M)	39	3.22	5.6
Low (L)	29	3.00	5.4
Nonvirulent (N)	10	2.08[a]	4.4[b]

[a] In nonvirulent cultures the members of flagella per cell were significantly less than for high and medium degrees of virulence (odds —>100:1) and for low (odds —>20:1).

[b] In terms of the length of the bacterial cells of the respective cultures. See text for details. The length of flagella in (N) cultures was significantly less than for (H) and (M) virulent cultures (odds —>20:1) and approximately significant for (L) (odds about 20:1).

cultures of high virulence and least for the nonvirulent. One highly virulent single cell culture (Ontario 3b) had the highest average number of flagella per cell, or 6.6, whereas 1 highly virulent parent culture (New Zealand 1) after attenuation had the least, or 1.0.

Table 4 shows that the cultures high and medium in virulence had significantly more flagella per cell than those low in virulence and nonvirulent. Based on observations of active cultures, the highly virulent cultures always seemed to possess most rapid motion at the 24-hour stage, at the time the flagella staining preparations were being made. Over half the nonvirulent cultures had less than 1.9 flagella per cell whereas the average number of flagella per cell was 3.1 for all 106 cultures examined.

Length of flagella in relation to virulence.—The length of flagella for the cultures examined seemed to bear some relation to virulence because as virulence decreased the proportion of cells with short flagella increased. Table 4 demonstrates that the length of flagella of nonvirulent cultures was significantly less than for cultures high or medium in virulence but on the border of significance for cultures low in virulence. The difference in length of flagella among cultures possessing different degrees of virulence was not significant. To compute length, all individual cells together with their flagella were measured in 3 microscopic fields; then the average of the flagella lengths was divided by the average of the cell lengths to give the factor in Table 4.

PHYSIOLOGY.—*Virulence in relation to physiological behavior.* — Numerous physiological tests were completed, including the utilization of carbon and nitrogen sources in relation to virulence, by early 1937. Decline or loss of pathogenicity was never correlated with carbon or nitrogen utilization, but variability in extent of activity was encountered as between cultures in most of the tests.

None of the cultures produced indol. None reduced nitrate to nitrite or nitrite to ammonia, but all produced ammonia. All fermented litmus milk. None hydrolyzed starch. All 136 cultures liquefied gelatin and while there was much variation it seemed unrelated to virulence, with nonvirulent cultures producing as much or more abundant liquefaction than those with high virulence.

Concerning carbon and nitrogen metabolism the results are indicated as follows: 1) Chemicals utilized by all, or practically all, the cultures are italicized; 2) chemicals fermented by some but not all cultures appear in bold face; and 3) unfermented chemicals appears in ordinary type. Below are enumerated the principal sources tested: Hexoses—*dextrose, galactose, levulose,* **mannose**; pentoses—*arabinose,* xylose, rhamnose (+8); disaccharides—**cellobiose** (+35), **lactose, maltose,** *sucrose, trehalose* (−6); trisaccharides—**raffinose,** *melezitose* (−7); polysaccharides—cellulose, *dextrin,* **glycogen, inulin,** starch; glucosides—*arbutin, amygdalin, phloridzin, salicin;* polyhydric alcohols—dulcitol, erythritol, **glycerol,** *mannitol,* **sorbitol** (+77), **inositol** (+80); amino acids—*asparagin,* **alanine, leucine, proline, glycine,** valine, *cystine,* cysteine, **tyrosine,** tryptophane; organic acids—acetic, benzoic, *citric,* **formic** (+44), **glycollic** (+47), **lactic** (+21), *malic,* maleic, malonic, *hippuric,* oxalic, propionic, salicylic, *succinic,* **tartaric** (+13); nitrogen compounds—*sodium nitrate, potassium nitrate, ammonium nitrate, ammonium sulfate, uric acid, urea,* **dicyandiamide** (+13), *oxamide, acetamide,* succinimide, *asparagine, barbital sodium, guanidine carbonate, d-glutamic acid, yeast extract.*

For carbohydrates, organic acids, and nitrogen utilization different standard synthetic media were employed. The formula for carbohydrates was: $MgSO_4$, 0.2 gm.; $NH_4H_2PO_4$, 1.0; KCl,0.2; carbon source, 10.0; water, 1000cc. For organic acids: $NaNH_4HPO_4$, 0.5 gm.; NaH_2PO_4, 0.17; KCl, 0.2; acid or sodium salt, 1.2; water, 1000cc. For nitrogen utilization: K_2HPO_4, 0.2gm.; $MgSO_4$, 0.2; NaCl, 0.2; dextrose, 5.0; nitrogen source, 5.0; water, 1000cc. The carbohydrate medium was far from satisfactory. Ark's (1) medium ($MgSO_4$, 0.3gm.; K_2HPO_4, 2.0; $(NH_4)_2HPO_4$, 6.0; water, 1000cc. plus 1 per cent of various carbohydrates) with more buffering capacity favored better growth especially in the tests on mannose, cellobiose, maltose, lactose, and arbutin. The degree or extent of fermentation seemed never to be correlated with virulence and oftentimes attenuated cultures seemed most active.

GENERAL.—*Parent cultures versus single cell progenies in relation to virulence.*—When parent cultures and their single cell progenies were classified on the basis of virulence (Table 5), there was considerable variability in successive years, illustrated for 1934 and 1935. Neither the parent (single colony) nor the progeny (single cell) cultures as a whole were stable. On the basis of the previous cultural purification studies it seemed probable that the majority of the single colony cultures finally employed were actually single cell cultures.

Referring to the 1933-37 high viruence summary column in Table 1, of the 8 cultures all but 2 were

TABLE 5.—*Classification of parent cultures and their single cell progenies on the basis of virulence to show variability on successive years—1934 and 1935*

	Virulence (Degree)	36 Parent Cultures number	85 Progeny Cultures (S. C.) High	Medium	Low	Nonvirulent	Total
Year	High	8	10	8	1	0	19
	Medium	23	10	35	3	4	52
1934	Low	2	3	3	0	0	6
	Nonvirulent	3	1	3	2	2	8
	High	13	10	8	4	7	29
	Medium	9	8	7	4	6	25
1935	Low	10	4	5	3	11	23
	Nonvirulent	4	2	1	2	3	8

single cell cultures; of the 15 which made only 1 departure from high virulence, all but 4 were single cell cultures. This is considered strong evidence that single-cell cultures are more stable than single colony cultures which are not of single-cell progeny.

Growth rates in relation to virulence.—In studying growth rates in relation to virulence (8), the loss of pathogenicity in *Erwinia amylovora* seemed to have an effect on generation time. A culture from Virginia (Va3) illustrates this point. For example, single cell progeny Va3a was low in virulence in 1936 and became nonvirulent or attenuated in 1937, whereas single cell progeny Va3c remained high in virulence throughout. Loss in virulence by Va3a seemed to be correlated with a shortening of generation time (77 to 72 minutes), whereas Va3c's generation time was slightly increased (80 to 82 minutes). Of the phytopathogenic bacteria thus far examined, generation times seem to range between 1 and 3 hours. In the above study 11 species showed generation times ranging from 55 to 155 minutes. These could be placed in 2 groups, one requiring between 1.0 and 1.5 hours and the other between 2.0 and 2.6 hours. Of course, the temperature of 30°C. which was employed is another factor to consider in making an evaluation. Also the use of carbon sources and special media have an effect on generation time because it may be increased or decreased depending on individual cultural preference.

Growth status of host plant in relation to disease susceptibility or resistance.—When attempting to evaluate host susceptibility it seems desirable to develop a readily reproducible nutritional method for achieving uniform vigorous growth. Obviously the growth status of a host is extremely important in evaluating susceptibility or resistance to disease. As an illustration of the fact that different hosts have different levels of susceptibility, Ark (1) found an R type of his isolate 50 to be avirulent for the shrub hosts tested whereas it was slightly virulent to pear fruits and succulent shoots.

In 1932, in attempting to evaluate the blight resistance of a pear seedling (Richard Peters) at State College, Pa., it was found to be essentially immune under field conditions by wound inoculation of succulent shoots. At Ithaca, N. Y., potted Richard Peters pear trees were subjected to high temperatures. This altered the C/N nutritive ratio through high respiration and resulted in depletion of carbon, making this highly resistant variety susceptible to shoot blight. Thus the high resistance was broken by altering the growth status of the host by means of heat.

In 1937, employing the full set of 136 cultures and numerous young trees propagated from the original Richard Peters seedling, it was found that the new variety was essentially immune from shoot blight and resistant to blossom blight (30 per cent infection) under field conditions at State College, Pa. Undoubtedly growth status of the host plays an important role in the incidence of all bacterial diseases of plants, especially when unusual outbreaks occur.

In the case of fire blight, the most susceptible part of a blight resistant tree (Richard Peters) was the blossoms. Careful tests on susceptibility of succulent shoots on small seedlings at Geneva, N. Y., on young trees before bearing, and on bearing trees, indicated that the small seedlings might be slightly more susceptible to shoot inoculation because of their overall more succulent growth status. This discussion is conditioned on the basic assumption that young stem meristems of a given species are virtually alike physiologically regardless of age of plant.

CONCURRENT INVESTIGATIONS.—*Infectivity of the fire blight organism.*—Studies were conducted on the minimum of inoculum required for infection in the various courts such as blossoms, shoots, and fruits. Single cells of the first blight organism were found to be capable of blighting apple blossoms when introduced into the nectary (5). Nectar removed aseptically from apple blossoms was found to be a suitable culture medium for growing single cell cultures of *E. amylovora.* That study demonstrated convincingly the great potential for spread of infections during the blossom blight phase of fire blight (6). Incidentally, though previously unreported, the sugar concentration of the nectar under highly humid conditions ranged from 0.5 to 3.0 per cent and the principal sugar contained in the nectar was sucrose.

Electrokinetic studies in relation to virulence.—Pathogenicity or virulence of the fire blight organism was found not to be correlated with electrophoretic velocity (4). The cultures used were selected from the study on variability. Mobility-pH curves at constant ionic strength were determined for 2 organisms (*E. amylovora* and *Bacterium stewartii*) and were dis-

tinctive for each species. Based on preliminary tests, other phytopathogenic species also seemed to possess a distinctive curve of a suggestive specific character, inviting further exploration and investigation.

Knaysi (12), in reviewing other work on the relation between electrophoretic velocity and virulence, and also the above work where no correlation was found between electrophoretic velocity and virulence of strains of *E. amylovora* and *B. stewartii*, suggests: "Obviously virulence is a complex function of a number of variables one of which, perhaps an important one, is the zeta potential of the cell." Zeta potential is the electrostatic charge on the surface of a particle and peculiar to it.

DISCUSSION AND CONCLUSIONS.—In this country the fluctuations in prevalence and sporadic character of fire blight are known to be variable from place to place and from year to year. The story under California conditions was clearly demonstrated by Ark (1) who offered an explanation on the inception of blight infection and on its subsequent rate and degree of spread. The roles of the principal infection court (nectary) and of rainfall or high humidity (to lower nectar concentration) would undoubtedly favor not only the rapid increase of virulent forms but also increase the virulence of weakly pathogenic forms. However, the story would not be complete without taking into account the role of temperature in insect flight during epiphytotics of blossom blight.

When isolated from old dormant cankers most of the cultures were rough (R) and low in virulence; when isolated from young infections practically all colonies were smooth and high in virulence. Passage of low-virulent cultures through suscept tissue usually restored virulence, whereas loss of virulence was frequently encountered when cultures were grown constantly on culture media, as in this study.

This report is largely concerned with cultures that were isolated, purified, grown on standard nutrient broth media for 5 years, and never revitalized by passage through suscepts except for evaluating virulence. Some cultures upon attenuation lost virulence permanently because no effort was made to rejuvenate them. Some cultures remained consistently high in virulence. The majority of cultures, however, demonstrated considerable variability even though grown under regular cultural conditions, from high virulence to practically total loss of virulence (attenuation) and recovery. The least variability or greatest stability was obtained for single-cell cultures. The fact that some single colony mother cultures possessed relatively high stability in virulence and cultural characters suggests the possibility that they were probably single-cell cultures to begin with. It should be borne in mind that during the first 8 months of the study while the cultures were being assembled and purified, the original 62 mother cultures had been reduced to 49 because of contamination or loss of virulence.

This report demonstrates how the purest of cultures of the fire blight organism vary in behavior under regular conditions of artificial culture. The unreported studies on cultural variability, some of which have been mentioned, confirmed those of Ark (1). This report suggests the need for more detailed studies on synthetic media for evaluating nutrition and the value of more detailed studies on more of the phytopathogenic species.

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TWO WATERMELON MOSAIC VIRUS STRAINS FROM CENTRAL FLORIDA [1]

C. W. Anderson [2]

SUMMARY

Two strains of watermelon mosaic virus (*Marmor citrulli*) infected 12 cucurbitaceous species, and 1 strain was recovered from a thirteenth naturally infected species. Neither strain infected *Momordica charantia*, *Nicotiana, tabacum*, *Capsicum frutescens*, or *Zinnia elegans*. Both strains were transmitted by *Myzus persicae*, and both were inactivated in extracted sap by 60°C./10 min. or by 10 days' aging. Both withstood approximately 1/10,000 dilution. Immunological studies were inconclusive. The yellow strain differed from the type in the symptoms induced in all 12 species and proved slightly less stable in tests of its physical properties. It induced symptoms in *Cyclanthera pedata* and proved lethal to Hale's best cantaloupe.

Two cucumber varieties appeared tolerant to the southern strain of cucumber mosaic virus but not to watermelon mosaic virus. Watermelon mosaic virus was compared with 3 other cucurbit viruses. Possible control methods were briefly discussed.

Several viruses can infect cucurbits. Freitag (14) recently stated that 7 viruses attack cucurbits in California; in Florida, Anderson (4, 6) described watermelon mosaic virus but presented no illustrations of its symptoms. This report extends the list of species known to be susceptible to watermelon mosaic virus, describes a second strain of the virus, and illustrates the symptoms induced by both strains.

MATERIALS AND METHODS.—The 2 virus strains studied were the type strain of watermelon mosaic virus (4, 5, 6, 7), obtained at Coleman in 1950 from Yellow summer crook-neck squash, and the yellow watermelon mosaic virus strain (7) obtained from the same host at Alachua in 1951.

The carborundum method of mechanical inoculation was used. Usually, the cotyledons of seedling plants were inoculated. Symptoms usually developed in the first or second leaf formed following inoculation. Plants of most species remained healthy if they produced 4 leaves after inoculation without showing symptoms. From 2 to 29 plants of each type were inoculated with each strain.

Attempts were made to recover each strain from each plant type inoculated. In each attempt, 2 Early prolific straight-neck squash (*Cucurbita pepo melopepo* Alef.) plants were inoculated with sap from a single plant source of inoculum. Unless otherwise noted, each strain was recovered from each susceptible plant species at least twice, and from each susceptible variety at least once.

Some parallel inoculations—in which similar plants of a given cucurbit type were separately inoculated with the 2 strains on the same day and reared under identical conditions—were made with each of the 23 cucurbitaceous types except *Momordica charantia* L. The symptoms induced by the type strain in the parallel inoculations invariably differed from those caused by the yellow strain in the same way as in any separate inoculations made.

RESULTS.—*Hosts and symptoms.*—Both virus strains induced systemic symptoms in plants of the cucurbitaceous types *Cucurbita pepo melopepo* Alef. (7 varities); *C. pepo medullosa* Alef.; *C. maxima* Duchesne; *C. moschata* Duchesne; *C. okeechobeensis* (Small) Bailey; *Cucumis sativus* L.; *C. melo* L. (Hale's best cantaloupe); *Citrullus vulgaris* Schrad. (Cannon ball watermelon); *C. vulgaris citroides* Bailey (Colorado preserving citron); *Melothria pendula* L.; *Lagenaria siceria* Standl. (= *L. vulgaris* Ser. = *L. leucantha* Rusby); *Luffa cylindrica* Roem.; and *L. aegyptiaca* Mill. (= *L. acutangula* Roxburg). The yellow strain also induced systemic symptoms in plants of *Cyclanthera pedata* Schrad. Each strain was readily recovered from each plant type, except that recovery of the yellow strain from cantaloupe plants was not attempted. Moreover, the yellow strain was recovered from *Luffa cylindrica* plants on only 1 of 8 squash plants. Neither strain induced symptoms in or was recovered from plants of the cucurbit *Momordica charantia*, or of the non-cucurbits *Nicotiana tabacum* L., *Capsicum frutescens* L., and *Zinnia elegans* Jacq. The yellow strain was recovered from naturally infected plants of *Cucumis anguria* L.

The incubation periods of both strains varied from about 4 to 12 or 15 days during the summer, but in winter longer incubation periods were sometimes noted in plants of watermelon and of *Cucurbita okeechobeensis*, particularly.

The type strain caused mild chlorosis, stunt, distortion, and mottle in plants of the *Cucurbita* species (Fig. 1.B), cucumber, watermelon, citron, *Melothria*, and *Lagenara*, and also in cantaloupe (Fig. 1.D-F). The mottle usually consisted of green bands along the veins or of raised green blisters and mildly chlorotic interveinal areas. The leaf apices often formed long narrow, sometimes twisted projections (shoestring), especially in the commercial varieties of *Cucurbita pepo*. Symptoms were very mild or absent in plants of *C. okeechobeensis*. Plants of the *Luffa* species de-

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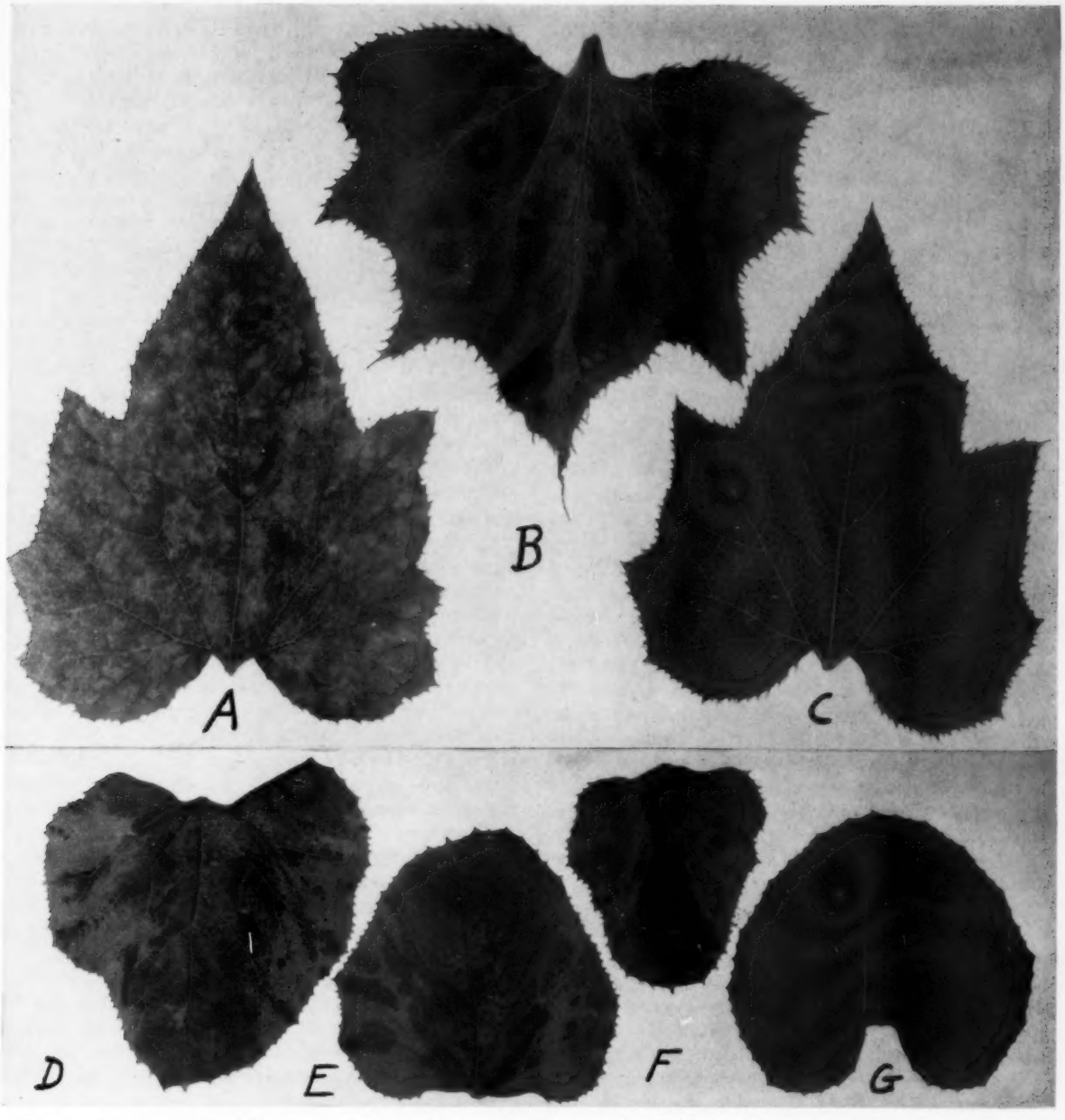

FIG. 1. Symptoms caused by watermelon mosaic virus in Early prolific straight-neck squash and Hale's best cantaloupe leaves. A) Yellow strain in squash. B) Type strain in squash. C) Healthy squash. D, E, F) Type strain in cantaloupe. G) Healthy cantaloupe.

veloped small, mildly chlorotic spots or rings, and a mild vein banding which differed from that seen normally in the leaves of *L. cyclindrica* primarily in being irregular and intermittent.

The yellow strain induced more chlorosis, stunting, and distortion in plants of the *Cucurbita* species, (Fig. 1,A), cucumber, *Citrullus*, *Melothria*, and *Lagenaria* than the type. The yellow strain was more likely to induce chlorotic rings and spots, but less likely to cause blisters or shoestring. It induced definite symptoms in plants of *Cucurbita okeechobeensis* in winter. Cantaloupe plants displayed a dull general chlorosis of both inoculated cotyledons and systemically affected leaves accompanied by rolling. The leaf veins were yellowish or brownish, and the leaves were ruffled and stunted. These symptoms were followed by general wilting and death. The yellow strain induced small but definite yellow spots or intermittent vein yellowing in plants of the *Luffa* species and larger, more diffuse, but less striking chlorotic spots in those of *Cyclan-*

thera pedata. The usual mottling, distortion, and stunting was virtually absent in plants of both *Luffa* and *Cyclanthera*.

Physical properties.—Early prolific straight-neck squash (*Cucurbita pepo melopepo* Alef.) plants were used as source and test plants in determining the physical properties of the 2 strains. Distilled water was used to dilute infectious sap. The thermal death points of both strains lay between 55 and 60°C./10 minutes. The tolerance to dilution of the type strain lay between 1/10,000 and 1/30,000. Its resistance to aging *in vitro* (18–21°C.) lay between 9 and 10 days. It remained active in frozen sap (–17°C.) for 364 days on 1 occasion.

The yellow strain withstood 1/1,000 dilution fairly readily. In 1 test it withstood 1/30,000 dilution, but it was usually less resistant to dilution than the type. The yellow strain withstood 6 but not 7 days' aging, and in 1 test remained active in frozen sap for 175 days. Its physical properties were similar to those of the type but the yellow strain proved slightly less stable than the type.

Both strains lost infectivity more rapidly in tests of their resistance to aging and to freezing when their infectivity was repeatedly tested at short intervals than when relatively few inoculations were made at longer intervals. Alternate freezing and thawing may have hastened virus inactivation in frozen sap, but this possibility does not explain the results obtained in tests of resistance to aging. Usually, the type strain remained infective for 4–6 tests if these were completed within 140 days of the day on which the sap was first frozen. This strain was usually inactivated in less than 4 tests if the period covered was much more than 140 days. Frozen sap containing the yellow strain was always inactivated by the third test. The type strain can usually be stored in crude frozen sap for 140 days. It is not advisable to maintain the yellow strain in frozen sap.

Vectors.—Anderson (6) found that *Aphis gossypii* transmits the type strain, probably in the typical non-persistent manner. Aphids (*Myzus persicae* Sulzer) were fasted for 12–25 hours, given an acquisition penetration period of 18–30 seconds on diseased straight-neck squash or *Melothria* plants, and transferred to healthy crook-neck or straight-neck squash plants for 25–55 minutes, using 1 aphid per test plant. The type strain was transmitted to 6 of 20 squash plants; the yellow strain, to 9 of 31 plants. Fifty-one control plants remained healthy, although aphids from the same colonies, similarly treated except that they were merely placed on the source plants momentarily and removed before they had attempted to penetrate the source plants with their stylets, were placed on 31 of the control plants. Both strains are aphidborne viruses, probably of the non-persistent group.

Immunological studies.—Anderson (4, 5) described the results of cross-protection tests between the type strain of watermelon mosaic virus and the V1 isolate of cucumber mosaic virus, an isolate representing southern cucumber mosaic virus (*Marmor cucumeris commelinae* H.). He concluded that watermelon mosaic virus is unrelated to the latter. In the present work, cucumber plants systemically invaded by the type strain of watermelon mosaic virus developed no additional symptoms in 20–24 days following inoculation with the yellow strain, although healthy cucumber plants of the same age developed symptoms of the yellow strain in 8–10 days. Attempts to recover the yellow strain on plants of *Cucurbita okeechobeensis* provided unsatisfactory results, as symptoms of both strains were extremely mild under the warmer environmental conditions of these tests. However, the yellow strain appeared to have been recovered from some of the cucumber plants which had been inoculated with it only. No evidence that it had been recovered from plants inoculated only with the type, or with the type and later with the yellow strain, was obtained. Possibly, the type protected cucumber plants against systemic invasion by the yellow strain, but adequate proof is lacking.

Discussion.—*Relationships between the type and the yellow strain.*—Both strains infected 22 cucurbitaceous plants, including 12 species (*Cucurbita pepo, C. maxima, C. moschata, C. okeechobeensis, Cucumis sativus, C. melo, Citrullus vulgaris, Lagenaria siceria, Cyclanthera pedata, Luffa cylindrica, L. aegyptiaca*, and *Melothria pendula*). Neither strain induced symptoms in nor proved recoverable from plants of the cucurbit *Momordica charantia* or the non-cucurbitaceous species *Nicotiana tabacum, Capsicum frutescens*, or *Zinnia elegans*. Both strains proved transmissible by *Myzus persicae*, apparently in the non-persistent manner. The thermal death points of the isolates were identical, and their other physical properties were similar. The type strain may have protected cucumber plants from systemic invasion by the yellow one, although conclusive proof is lacking. The symptoms induced by the type strain differed from those of the yellow one in all 22 susceptible cucurbit varieties (12 susceptible species), even when inoculations were made under identical conditions. Therefore, both isolates are strains of watermelon mosaic virus (4, 5, 6, 7).

Relationships between the watermelon mosaic virus and other viruses.—Many viruses affecting cucurbits have wide host ranges and infect tobacco. Watermelon mosaic virus seems unrelated to any of these. A few viruses, such as the sap-transmissible virus isolated from necrotic ring spot cherry trees and other stone fruits (8, 15, 17, 24) and papaya mosaic virus (1, 2, 9), occur naturally in plants of other families but infect cucurbits although their known host ranges are restricted. There is no evidence that watermelon mosaic virus is related to any of these.

Of several viruses occurring naturally in cucurbits and having restricted host ranges, only cucurbit mosaic virus (*Marmor astrictum* Holmes) (3, 10, 18, 22, 23), muskmelon mosaic virus (*Marmor melonis* Rader et al) (19), and bottle gourd mosaic virus (*Cucumis*

TABLE 1.—*A comparison of watermelon mosaic virus with 3 other cucurbit viruses*

Virus	Vectors	Physical properties[a] Thermal Death Point (°C./10 min.) +	−	Longevity *in Vitro* +	−	Reactions of selected plant species[b] Cucurbita pepo	Citrullus vulgaris	Momordica charantia	Nicotiana *tabacum*
Marmor astrictum	NA[c]	80	98	3+Mo.		N-T	S-T	T	N-LT
Marmor melonis	?	60	62	10Da.		S	N		N
Cucumis virus 3	?	55	60	4½Hr.	6Hr.	S	S	S	N
Marmor citrulli	A	55	60	6Da.	10Da.	S	S	N	N

[a] The properties of *Marmor astrictum, M. melonis,* and *Cucumis* virus 3 were determined in hosts other than squash. The figures listed are the extremes and are not necessarily those reported by any one writer for any one strain.

[b] The meanings of the symbols are as follows: S = Systemic symptoms induced; T = Latent infection; L = Local symptoms only induced; LT = Local latent infection only; N = Immune; − = Or

Examples: N-T indicates that the plant is reported immune to some strains but that latent (masked) infection occurs with others. S-T indicates that some strains induce systemic symptoms but others are latent.

[c] The symbols in this column indicate: NA =Aphid transmission attempted with at least one strain with negative results; A = Transmitted by aphids; ? = No record of attempted insect transmission.

virus 3 Vasudeva & Lal) (21) are sufficiently described to justify detailed comparison with watermelon mosaic virus. The data (Table 1) indicate that watermelon mosaic virus resembles *Cucumis* virus 3 most closely. The 2 watermelon mosaic virus strains described in this report differ from *Cucumis* virus 3 in their failure to infect *Momordica charantia,* in physical properties, and in the type of symptoms induced in certain hosts. Since the properties of *Cucumis* virus 3 were studied in plants of *Lagenaria siceria* rather than in squash, it is possible that greater similarities could be found. For the present, watermelon mosaic virus is considered a virus distinct from *Cucumis* virus 3 because of the magnitude of the differences in physical properties and the difference in host range.

Freitag (12, 13, 14) lists 6 viruses other than cucumber mosaic virus as occurring in cucurbits in California. Detailed descriptions of these are not yet available, but the viruses (14) of squash mosaic, wild cucumber mosaic, and cucurbit ring mosaic (muskmelon mosaic virus Freitag) are transmitted by beetles but not by aphids. They are therefore unrelated to watermelon mosaic virus. The principle cucurbit virus of California's Imperial Valley is aphid-transmissible and capable of infecting squash, melon, cucumber, and watermelon plants (11, 16). It is seed-borne, and squash mosaic virus was believed to be the cause (16). Freitag (14), however, indicates that this cannot be squash mosaic virus since he has demonstrated that the latter virus is transmitted only by beetles and not by aphids. The relationship between watermelon mosaic virus and the principal cucurbit virus of the Imperial Valley has not been indicated.

Technical descriptions.

Marmor **citrulli** sp. nov.

Common name: Watermelon mosaic virus.

Origin: *Cucurbita pepo melopepo* Alef. from Coleman, Florida, May, 1950.

Host range: All tested cucurbits (22 varieties, 12 species) except *Momordica charantia.* All tested non-cucurbitaceous species (4) including *Nicotiana tabacum* immune.

Differential symptoms: Mottle, mild chlorosis, green vein banding, raised green blisters, distortion, and shoestring in most suscepts. Latent in *Cyclanthera pedata;* nearly latent in *Cucurbita okeechobeensis, Luffa cylindrica,* and *L. aegyptiaca.*

Transmission: Transmitted by aphids, apparently in typical non-persistent manner, and by sap.

Properties (determined in *Cucurbita pepo melopepo*): Thermal death point, between 55 and 60°C./10 min.; tolerance to dilution, between 1/10,000 and 1/30,000; resistance to aging, between 9 and 10 days (18-21°C.).

Marmor citrulli ***flavidanum*** var. nov.

Common name: Yellow watermelon mosaic virus.

Origin: *Cucurbita pepo melopepo* Alef. from Alachua, Florida, September, 1951.

Host range: Identical with that of *M. citrulli* so far as tested.

Differential symptoms: Symptoms similar to those of *M. citrulli,* but yellow strain causes more chlorosis, stunting, and distortion in most species and is more likely to cause small yellow rings and spots, but less likely to induce shoestring. Causes definite disease in *Cucurbita okeechobeensis* in winter, mild disease consisting of diffuse chlorotic spots in *Cyclanthera pedata,* and small yellow spots or intermittent yellowing of the veins in 2 *Luffa* species. Lethal to Hale's best cantaloupe.

Transmission and physical properties: Similar to those of *M. citrulli.*

Immunological reactions: *M. citrulli* may prevent this strain from invading cucumber plants, but adequate proof is lacking.

Control.—Wellman indicated that control of cucumber mosaic virus in central Florida depends largely on the elimination of the perennial weed, *Commelina nudiflora.* Anderson (6, 7) found that the perennial *Melothria* species are important sources of watermelon mosaic virus in the area but failed to obtain evidence of seed transmission of the virus. Certain cucurbit viruses, including *Marmor melonis* (19), are seed-borne, and Anderson (6) briefly reviewed reports con-

cerning the possible seed transmission of various viruses in cucurbits. Stoner (20) found that the cucumber mosaic virus-resistant Ohio MR 17 and Niagara cucumbers were readily susceptible to the southern strain of cucumber mosaic virus in the greenhouse. Limited tests by the writer, in which these cucumbers and Marketer cucumber were inoculated in the greenhouse with the V1 isolate (southern strain) of cucumber mosaic virus or with the type strain of watermelon mosaic virus, confirmed this finding but indicated that the reaction of the cucumber mosaic virus strain on Ohio MR 17 and Niagara was less severe than that on Marketer. However, watermelon mosaic virus produced reactions of approximately equal severity on all 3 varieties.

The above considerations illustrate the added difficulties produced by the occurrence of distinct viruses in devising control methods against mosaic diseases in cucurbits. Weed control, use of resistant or tolerant cucurbit varieties, and use of disease-free seed are or may become important in controlling cucurbit viruses in central Florida. The control of *Melothria* is a different problem from that of *Commelina*, since the former commonly grows in uncultivated areas whereas populations of the latter build up on cultivated land.

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DYNAMICS OF DEPOSITION AND TENACITY OF FUNGICIDES[1]

Saul Rich

SUMMARY

In field spraying with Bordeaux, there were smaller increments of initial spray deposit with increasing spray concentrations. This relationship is thought to depend on adsorption of the spray particle by the leaf surface. As spray concentration increased, the adsorptive capacity of the leaf surface approached saturation. The unadsorbed particles were supposedly lost in the run-off liquid. The Bordeaux data fitted very closely the equation for Langmuir's adsorption isotherm.

The build-up of zineb deposits, however, was approximately proportional to the concentration of spray material in the tank. This linear relationship probably exists because adsorptive forces are not involved in the formation of zineb deposits.

Smooth celery leaves retained more zineb deposit from the spray liquid than did hairy bean leaves. Both types of foliage retained about the same amount of Bordeaux deposit from the spray liquid. These differences are attributed to differing areas of spread. A zineb suspension spreads more readily on celery than it does on beans. Bordeaux spreads equally well on both types of foliage.

During the weathering of dried Bordeaux deposits, the larger the initial deposit, the smaller is the percentage lost. This indicates that the dried Bordeaux particles cohere more strongly to each other than they adhere to the leaf surface. Dried zineb particles weather in an opposite fashion to Bordeaux. The larger the deposit of zineb, the greater is the proportion of its mass lost by weathering. Dried zineb particles must, therefore, adhere more strongly to the leaf surface than they cohere to each other. Regardless of the material used, deposits on bean leaves weathered more slowly than deposits on celery leaves.

Bordeaux and zineb were found to have 2 physical differences which could account for their different behaviors in deposit build-up, and in residue weathering. 1) Bordeaux particles carry a positive electrokinetic charge, whereas zineb particles are negatively charged. 2) Bordeaux suspensions are hydrophilic, whereas zineb suspensions are hydrophobic.

The characteristics of zineb deposition and weathering were found to be the same, whether the original spray was a formulation of a zineb wettable powder, or a tank-mix of nabam plus zinc sulfate.

In general, deposit build-up and weathering of dried residues are greatly influenced by the type of material being applied, and the type of foliage being sprayed.

There are 2 main factors determining the amount of spray residue remaining on the sprayed plant to provide protection against plant pathogens. The first factor is the amount of material which sticks to the leaf surface during spraying. The second factor is the amount of residue which will remain after weathering.

The application of spray materials to a leaf surface may be divided into 2 phases: pre-run-off, and run-off. During the pre-run-off stage, the factors involved in the build-up of spray deposit[2] are basically 1) the concentration of spray in the tank, 2) the volume of spray, and 3) the attraction of the spray droplets for the sprayed surface and for each other. After run-off begins, the original contributions of these 3 basic factors are modified. The modifying influences are 1) the attraction of the spray particles for the sprayed surface, 2) the attraction of these particles for each other, and 3) the volume of run-off. It has generally been assumed that simply increasing concentration in the spray tank results in a proportionate increase of spray deposit on the leaf surface. Mader and Blodgett (16) working with Bordeaux on potatoes, concluded "that the amount of copper on plants was approximately proportional to the concentration of the mixtures applied." Horsfall (12), however, wrote that "deposit accumulates according to the logarithm of concentration."

The second important factor, weathering of the dried deposit, is strongly influenced by 1) the amount of the deposit, 2) the attraction of the dried spray particles for the sprayed surface, 3) the attraction of the dried spray particles for each other, 4) chemical changes brought about by weathering, and 5) intensity of weathering. Horsfall (12) reviewed the literature on the weathering of dried deposits and concluded that in the majority of cases resistance to weathering decreased as the size of deposit increased. Turner and Woodruff (22) demonstrated in the laboratory that relation of tenacity to size of deposit depends on the material being studied.

How then do spray deposits perform in the field? This paper will deal with field experiments showing the effect of spray concentration on the initial deposition and subsequent weathering of 3 commercially important spray materials: Bordeaux, a zineb preparation (Dithane Z-78), and tank mix zineb, i.e., nabam (Dithane D-14) plus zinc sulfate. Thoroughout this paper zineb will refer to Dithane Z-78. Dithane D-14 plus zinc sulfate will be referred to as the nabam mix. The materials were tested on plants with hairy leaves (beans) and with smooth leaves (celery).

MATERIALS AND METHODS.—Field experiments were accomplished in the randomized spiral plots described by Horsfall and Rich (13). Sprays were applied at 250 lb. pressure per sq. in. and 200 gal. to the acre. Pressure and gallonage were held constant throughout. Plots were sprayed once a week. Beans were

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[2] Deposit is defined as the amount of material which is retained by a sprayed surface after any run-off has ceased.

TABLE 1.—*Bordeaux deposits and residues on bean and celery foliage. Data expressed as μgm. of copper per sq. cm.*

Sampling date	Time of sampling relative to spray application	Concentrations of Bordeaux per 100 gal. of spray 1.0–0.5	1.4–0.7	2.0–1.0	2.8–1.4	4.0–2.0	5.6–2.8	8.0–4.0	11.2–5.6
					Bean foliage				
7-6	After	1.20	1.73	2.30	2.38	3.50	4.03	4.05	5.47
7-11	Before	0.65	0.87	1.82	2.65	3.18	4.15	5.37	5.84
7-14	After	2.29	2.78	4.54	4.34	6.47	7.39	6.56	8.16
7-19	Before	1.32	2.43	4.38	5.38	6.56	7.06	7.29	8.31
7-20	After	4.05	4.56	6.32	7.07	8.24	8.16	9.63	10.08
7-26	Before	1.50	1.71	3.44	5.83	8.26	9.69	11.66	11.16
7-27	After	2.64	4.22	5.49	7.98	10.17	12.60	13.89	16.83
8-2	Before	1.66	2.11	3.49	5.50	9.05	11.63	12.29	13.45
8-5	After	2.45	3.53	2.81	6.36	8.71	11.33	12.03	14.34
Average	Before	1.28	1.78	3.28	4.84	6.76	8.13	9.15	9.69
Average	After	2.53	3.36	4.29	5.63	7.42	8.70	9.23	10.98
					Celery foliage				
8-19	After	.....	1.92	2.28	2.51	2.87	3.30	3.69	3.91
8-25	Before	.....	0.89	1.48	2.95	4.25	5.04	6.09	6.34
8-26	After	.....	3.35	4.53	6.71	8.12	9.64	10.80	11.34
9-1	Before	.....	0.17	1.20	2.19	3.52	3.86	4.51	5.25
9-2	After	.....	3.43	3.77	5.67	7.15	7.72	9.10	10.40
9-7	Before	.....	1.40	2.68	3.32	4.29	5.60	5.91	8.66
9-9	After	.....	2.98	4.39	6.85	9.61	9.38	10.57	15.11
9-15	Before	.....	0.81	1.46	3.26	6.31	6.79	7.33	10.03
9-16	After	.....	2.39	4.51	6.29	6.06	10.96	15.03	15.14
9-22	Before	.....	1.03	1.88	3.08	3.15	6.84	11.80	14.26
9-27	After	.....	2.28	3.91	5.49	6.50	10.43	12.26	14.94
Average	Before	.....	0.86	1.74	2.96	4.30	5.62	7.13	8.91
Average	After	.....	2.72	3.90	5.59	6.72	8.57	10.24	11.81

sprayed 5 times and celery received 6 sprays. Leaf samples were taken immediately after each spray and again just prior to the succeeding spray. In sampling, approximately 1000 sq. cm. of leaves were taken from each of the 4 replicated plots. Leaf areas were measured photoelectrically, using a modification of the device described by Frear (5). After leaf measurements were taken, analyses for copper or zinc were made spectrographically.[3] Results are expressed as μgm. of copper or zinc per sq. cm. of leaf surface. It is not meant to imply that zinc is the active portion of the zineb molecule, but only that zinc may be used as an indicator of amount of residue. Zinc may or may not remain a part of the fungicidally active portion of the zineb molecule throughout the weathering process. Nevertheless, the deposit and residue data for zineb herein presented may serve as a model for a distinctive type of deposit and subsequent weathering of a spray material. Throughout this paper, deposit and residue[4] will be expressed in units (u.) of 1 μgm. of copper or zinc per sq. cm.

Because of the extraordinarily warm, dry summer (1949), no disease developed in any of these plots, so that correlative disease data are not available.

BORDEAUX EXPERIMENTS.—On beans, the Bordeaux dosage series began at 1.0–0.5–100 and increased by a factor of $\sqrt{2}$ up to 11.2–5.6–100, using fresh, commercial, spray-grade lime. The same series was applied to celery, except that the lowest dosage was omitted because of limited field space. Data are in Table 1.

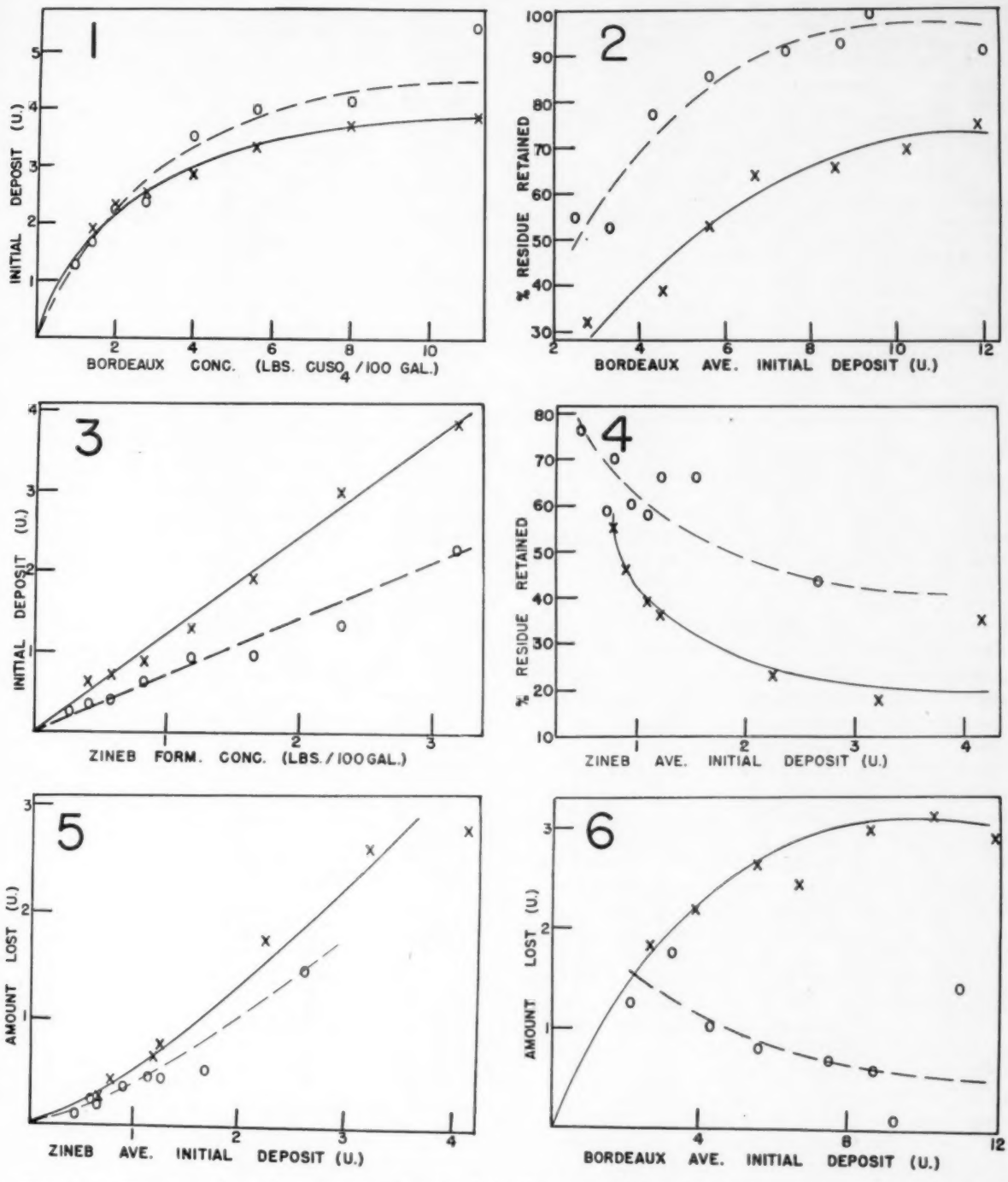
The effect of spray tank concentrations on build-up of deposit may be analyzed by examining the size of the deposits remaining directly after the first spray was applied to the beans on July 6. At 1 lb. of copper sulfate per 100 gal., 1.20 units of copper remained. Doubling the amount in the tank (2 lb. per 100 gal.) resulted in a deposit of 2.30 units of copper, or an approximate doubling of deposit size with tank concentration. Beyond this point, however, smaller and smaller additional increments were deposited on the leaf surface per unit increase in tank concentrations. For example, 4–2–100 Bordeaux gave a deposit of 3.50 u., while the deposit from 8–4–100 Bordeaux was 4.05 u., an increment increase of only 16%. This pattern was also followed by the residue of Bordeaux on celery foliage after the first spray was applied. When the data are graphed (Fig. 1) the patterns may be seen more clearly. The importance of run-off in developing this pattern will be presented in a later section.

Data for deposition of the first spray indicate that Bordeaux particles from spray droplets adhere only slightly better to a previously unsprayed hairy leaf than they do to a previously unsprayed smooth leaf. The average deposition for all concentrations after the first spray on beans was 3.34 u. On celery it was 2.93 u.

Once the spray has dried it becomes important to know if size of deposit is related to the amount lost by weathering, and if the type of leaf surface influ-

[3] The author wishes to thank Mr. W. T. Mathis of the Analytical Chemistry Department, The Connecticut Agricultural Experiment Station, for making the spectrographic analyses.

[4] Residue is defined as the amount of deposit remaining after weathering.



FIGS. 1–6. Deposits and residues expressed in units (u.) of 1 μgm of either metal per square centimeter of leaf surface; copper for Bordeaux and zinc for zineb formulation. Deposit on celery foliage indicated by "X"'s and solid lines; deposit on bean foliage indicated by "O"'s and broken lines. Fig. 1. Relation of Bordeaux concentration to deposition from first spray.—Fig. 2. Relation of size of Bordeaux average deposit to average per cent retained after weathering.—Fig. 3. Relation of zineb formulation concentration to deposition from first spray.—Fig. 4. Relation of size of zineb average deposit to average per cent retained after weathering.—Fig. 5. Relation of size of zineb average deposit to average amount of deposit lost by weathering.—Fig. 6. Relation of size of Bordeaux deposit to average amount of deposit lost by weathering.

TABLE 2.—*Increments of Bordeaux held by each type of foliage after each successive spraying, and average increments lost by each type of foliage between spray applications. Expressed as μgms of copper per sq. cm. of leaf surface*

	Copper sulfate concentration of Bordeaux in tank in lbs. per 100 gal.							
	1	1.4	2.0	2.8	4.0	5.6	8.0	11.2
				(Beans)				
Amount retained after 1st spray	1.2	1.7	2.3	2.4	3.5	4.0	4.0	5.5
Average of subsequent sprays	1.6	1.7	1.5	1.5	1.3	1.7	1.4	2.1
				(Celery)				
Amount retained after 1st spray	....	1.9	2.3	2.5	2.9	3.3	3.7	3.9
Average of subsequent sprays	....	2.1	2.7	3.2	3.2	3.8	4.3	4.5
				(Beans)				
Average amount lost	1.0	1.6	1.4	0.9	0.3	0.3	0.4	0.8
				(Celery)				
Average amount lost	....	1.7	2.2	2.7	2.6	2.9	3.2	2.8

ences weathering. The actual amounts lost per spray may be estimated by the differences between averages (Table 1). For beans, these differences from smallest deposits to largest are as follows: 1.25 u., 1.58 u., 1.01 u., 0.79 u., 0.66 u., 0.57 u., 0.08 u., 1.29 u. For celery, these differences in the same order are: 1.86 u., 2.16 u., 2.63 u., 2.42 u., 2.95 u., 3.11 u., 2.90 u. On beans in general the smallest deposits actually lost the largest increments through weathering. On celery, the amounts lost were fairly uniform, although the largest deposits lost more than the smaller deposits. The item of interest is actually how much residue remains to afford protection after a period of weathering. Percentage of residue retained was obtained from data averages as follows:

$$\frac{\text{Residue before spraying}}{\text{Deposit after spraying}} \times 100.$$

Fig. 2 shows the relationship between size of deposit and subsequent weathering. It is apparent with Bordeaux that the larger the deposit the greater is the percentage of residue retained. On celery, a 3.0 u. deposit retains 32 per cent of its mass after weathering, while an 11.0 u. deposit retains 73 per cent of its original mass. When these data are plotted on a semi-log grid, straight lines result. This demonstrates a proportionality between the percentage retained and the logarithm of deposit.

Weathering data show a distinct difference in the loss of deposit between the 2 different types of leaves. The hairy bean leaves lose dried Bordeaux at a much slower rate than do the smoother celery leaves. For comparable deposits, beans retain about 30 per cent more than do celery leaves. If this is so, why is the ultimate residue on both leaves approximately the same after a series of sprays have been applied? After 5 sprays the residues on celery averaged 8.62 u., while comparable data from beans averaged 8.44 u. There should be much more residue on the bean leaves if both types of leaf surfaces retain about the same amount of Bordeaux from the original spray and

TABLE 3.—*Zineb deposits and residues on bean and celery foliage. Data expressed as μgms of zinc per sq. cm.*

Sampling date	Time of sampling relative to spray concentration	Concentrations of zineb formulation per 100 gal. of spray							
		0.28	0.40	0.57	0.81	1.14	1.62	2.29	3.24
					Bean foliage				
7-6	After	0.24	0.35	0.41	0.66	0.93	0.96	1.27	2.16
7-11	Before	0.29	0.33	0.44	0.53	0.57	0.64	1.04	0.82
7-14	After	0.48	0.68	0.79	1.08	1.27	1.19	1.81	2.83
7-19	Before	0.38	0.31	0.46	0.72	0.82	1.09	1.48	1.90
7-20	After	0.46	0.74	0.84	1.00	1.43	1.66	2.06	4.06
7-26	Before	0.26	0.37	0.45	0.38	0.56	0.76	0.88	0.98
7-27	After	0.46	0.67	0.61	0.79	1.01	1.17	1.60	2.14
8-2	Before	0.37	0.44	0.49	0.51	0.66	0.65	0.97	0.91
8-5	After	0.46	0.58	0.66	0.97	0.94	1.08	1.45	1.81
Average	Before	0.32	0.36	0.46	0.54	0.65	0.79	1.09	1.16
Average	After	0.42	0.61	0.66	0.90	1.12	1.21	1.64	2.60
					Celery foliage				
8-19	After	.....	0.64	0.71	0.86	1.21	1.80	2.72	3.63
8-25	Before	.....	0.45	0.53	0.57	0.56	0.58	0.59	0.67
8-26	After	.....	0.81	1.08	1.37	1.86	2.45	2.82	4.39
9-1	Before	.....	0.38	0.44	0.46	0.42	0.56	0.57	0.68
9-2	After	.....	0.91	1.22	1.54	2.36	4.20	7.47	7.39
9-7	Before	.....	0.57	0.39	0.60	0.71	0.89	1.18	1.43
9-9	After	.....	0.64	0.60	1.08	1.29	1.71	2.29	3.79
9-15	Before	.....	0	0	0	0	0	0.23	0.47
9-16	After	.....	0.44	0.48	0.84	1.00	1.53	2.12	2.05
9-22	Before	.....	0.40	0.42	0.46	0.44	0.58	0.50	0.60
9-27	After	.....	0.49	0.63	0.85	1.16	1.83	1.75	3.53
Average	Before	.....	0.36	0.35	0.42	0.43	0.52	0.62	1.39
Average	After	.....	0.66	0.79	1.09	1.22	2.25	3.20	4.13

TABLE 4.—*Nabam mix deposits and residues on bean and celery foliage. Data expressed as μgms of zinc per sq. cm.*

Sampling date	Time of sampling relative to spray application	Concentrations of nabam mix expressed as lbs. of zineb formulation per 100 gal. 0.28	0.40	0.57	0.81	1.14	1.62	2.29	3.24
					Bean foliage				
7-6	After	0.55	0.64	0.74	0.81	1.18	1.75	1.97	2.06
7-11	Before	0.38	0.43	0.46	0.53	0.63	0.93	1.09	1.38
7-14	After	0.61	0.65	1.12	0.86	1.10	1.42	1.82	1.38
7-19	Before	0.54	0.52	0.40	0.51	0.69	0.94	1.25	1.25
7-20	After	0.78	0.91	1.17	1.56	1.75	2.97	3.14	3.85
7-26	Before	0.41	0.30	0.42	0.72	0.77	0.92	1.20	1.61
7-27	After	0.58	0.65	0.79	1.02	1.45	2.11	2.48	2.43
8-2	Before	0.47	0.47	0.42	0.49	0.83	1.02	1.03	1.26
8-5	After	0.55	0.56	0.87	1.12	1.04	1.54	2.11	2.59
Average	Before	0.45	0.43	0.43	0.57	0.73	0.95	1.14	1.38
Average	After	0.61	0.68	0.94	1.07	1.30	1.96	2.30	2.46
					Celery foliage				
8-19	After	.....	0.90	0.89	1.29	1.69	1.66	2.71	3.46
8-25	Before	.....	0.50	0.33	0.50	0.44	0.47	0.92	0.84
8-26	After	.....	1.14	1.22	1.81	1.98	2.15	2.82	4.23
9-1	Before	.....	0.57	0.55	0.64	0.71	0.81	0.94	1.11
9-2	After	.....	1.47	1.52	1.95	2.25	3.16	4.03	6.42
9-7	Before	.....	0.46	0.58	0.74	0.79	1.18	1.24	1.81
9-9	After	.....	0.88	0.86	1.07	1.44	2.06	3.13	3.64
9-15	Before	.....	0.00	0.00	0.00	0.23	0.29	0.43	0.61
9-16	After	.....	0.62	0.54	0.60	0.76	1.54	2.31	2.57
9-22	Before	.....	0.38	0.37	0.41	0.57	0.49	0.65	0.72
9-27	After	.....	0.49	0.65	0.67	0.95	1.15	2.40	3.09
Average	Before	.....	0.38	0.37	0.46	0.55	0.65	0.84	1.02
Average	After		0.89	0.95	1.23	1.51	1.95	2.90	3.90

much less is weathered from the bean leaves. The answer may be found by determining the actual increments of material held by each type of leaf after each spraying, and comparing them to the increments lost by each type of leaf between sprayings. The figures for deposit after the first spray represent spraying of a previously unsprayed leaf surface. Table 2 presents the averaged data.

Bean foliage lost an average of 0.70 u. of Bordeaux between sprays, while the average loss for celery was 2.60 u. The average increment gain per spray for bean leaves was 1.60 u., while for celery it was 3.38 u. It becomes obvious, therefore, that although the bean foliage loses much less dried Bordeaux residue by weathering than does the celery foliage, it also retains much less of the spray material subsequently applied to it. Immediately after each spray, consequently, both types of foliage have about the same amount of material at each given concentration of Bordeaux. After a period of weathering, however, the bean leaves have retained a much better protective covering. The practical implications of these phenomena will be discussed later.

ZINEB EXPERIMENT.—The dosage series for zineb also increased by the factor of $\sqrt{2}$. On beans, the dosage range extended from 0.28 to 3.24 lb. per 100 gal. The range was the same on celery except that it started at 0.40 lb. per 100 gal. Data are in Table 3. The concentrations of the nabam mix were adjusted to give exactly the same amount of active ingredient as the zineb concentrations. Data for the nabam mix (Table 4) are so similar to the zineb data that the descriptions and discussions concerning zineb deposits and residues may also be applied to the nabam mix. Fig. 3 presents the relation between dosage and deposit of zineb. Zineb particles apparently are deposited from the spray droplet in an amount arithmetically proportional to the concentration of the tank mix. This is true regardless of the foliage being sprayed. On celery the first spray deposited about 1.15 units per lb. of Z-78 per 100 gal. of spray. On beans only 0.75 unit was deposited per lb. of Z-78 in 100 gal. of spray. The bean foliage, therefore, retained less zineb deposit than did the celery. The answer undoubtedly lies in the difficulty of wetting the hairy bean leaves. The hairs probably cause air to be trapped under the spray droplets and prevent close contact between the liquid and the leaf surface. In the case of Bordeaux, the initial deposit on bean leaves was actually slightly larger than it was on celery leaves.

Once the zineb deposits have dried, their rate of weathering may be analyzed in the same manner as for Bordeaux. The actual amounts of zineb units lost by bean foliage from lowest deposit to highest deposit are: 0.10, 0.25, 0.20, 0.36, 0.57, 0.42, 0.55, 1.44. For celery, these figures are: 0.30, 0.44, 0.67, 0.79, 1.73, 2.52, and 2.74. From either foliage appreciably more zineb is weathered from the high deposits than from the low. These figures, expressed as percentage of deposit retained after weathering, are plotted in Fig. 4. It is seen that the weathering of these deposits, although logarithmic, is directly opposite to the pattern established for Bordeaux. The larger the zineb deposit the greater is the percentage lost by subsequent

weathering. Here again, however, there is a striking difference in influence of foliage on weathering. The hairy bean leaves apparently make the deposits more resistant to weathering. The celery leaves retain more of the material being applied, but again lose more of the dried deposit by subsequent weathering.

INITIAL RETENTION OF BORDEAUX.—One of the most interesting aspects of the Bordeaux data is the observation that the initial retention of deposit by the foliage increases as the logarithm of the concentration (Fig. 1). It would be of interest to know not only how this relationship develops, but also when the factors responsible are operative.

Deposit before run-off would be greatly affected by those factors which influence the volume of spray droplets which reach and stick to the leaf. Is the volume of spray liquid deposited influenced by the concentration of Bordeaux? Unless this volume is in some way controlled by the concentration of material, there should be a direct arithmetic relation between concentration of spray and the initial retention of deposit by the leaf before run-off. It was necessary to see if changing concentration in the spray tank varied the volume of liquid leaving the spray nozzles of the equipment used in these tests. This was done by trapping and measuring the liquid leaving the nozzles in a given spray time as Bordeaux concentration was changed in the tank. There was no difference in volume through the range of the Bordeaux concentrations used in our experiments (1.0–0.5–100 to 11.2–5.6–100).

Data were needed to separate pre-run-off from run-off phases of spraying. No attempt was made in these experiments to stop spraying before run-off. It was necessary, therefore, to find other data in the literature to illustrate the relation between concentration and deposition before run-off. Frear (6) presented laboratory data showing the effect of concentration on initial retention. He used Bordow, a proprietary copper material, sprayed on pyraline plates. Although he did not spray to run-off, his data indicate a definite logarithmic relation between concentration and initial deposit. Isely and Horsfall (14) obtained similar laboratory results using arsenical insecticides sprayed on wax surfaces. It is possible in certain instances, then, to have concentration logarithmically related to initial retention of spray material without run-off. Concentration, therefore, must have some influence on volume of spray striking and sticking to the leaf surface. The most obvious way that volume retained can be influenced by concentration is by the lowering of surface tension with increasing concentration. A phenomenon of this type was described by Fajans and Martin (4) who worked with additives which lowered the surface tension of spray mixtures. The volume of spray reaching the surface being sprayed was much less when the surface energy was lowered because of the shearing of liquids into more drops of smaller diameter when surface energy was lowered. The possibilities are increased therefore for 1) more droplets to evaporate before they reach the surface, and 2) more droplets failing to penetrate the air layers next to the surface of the target, as shown by Potts (21). In order to determine whether this phenomenon was operative in the case of Bordeaux, surface tension measurements were made on the various concentrations of Bordeaux used in the field experiments. The measurements were made with a Cenco-duNoüy Precision Tensionmeter. No appreciable change in surface tension was found with changes in Bordeaux concentrations. Changes in surface tension were, therefore, eliminated as a factor in the production of the Bordeaux initial retention curve.

Another possible explanation for a logarithmic relation between concentration and volume deposited before run-off may be the spreading of the spray cone with increasing concentration of spray mix. Increasing the amount of material in the spray liquid may possibly increase the angle of spread of the spray cone by increasing the friction between the sides of the nozzle aperture and the periphery of the spray stream as it leaves the nozzle. As the angle of the cone became wider a greater area would be covered by unit volume of spray. Hence a target with a constant area would receive less volume.

The following laboratory experiment was devised to test this hypothesis. A Hewlett spray nozzle (9) was placed 25 cm. from white Bristol board targets, and the air pressure was adjusted to 15 lb. per sq. in. The spray liquid was fed from a reservoir placed above the nozzle and the liquids agitated with a small laboratory stirrer. Diameters of the spray cones were marked and measured on the targets immediately after a 3-second spray period. Distilled water was compared to freshly prepared 11.2–5.6–100 Bordeaux, the highest Bordeaux concentration used in the field. Six targets were sprayed with each liquid. The diameters in centimeters are as follows: water, 19, 19, 19, 18, 18, 18½; for Bordeaux, 20½, 22, 20, 21, 20, 19. The average for water was 18½ cm.; for Bordeaux it was 20½ cm. This difference is statistically significant at the 1 per cent level. This experiment indicates that materials suspended in a spray stream tend to spread the spray cone. The amount of spread under the conditions described, however, does not seem sufficient to account for the logarithmic relationship between concentration and the volume deposited before run-off. Just what forces are responsible for this relationship remains obscure.

What is the situation when spraying is continued beyond run-off? As almost all field spraying continues beyond run-off, it is perhaps more applicable to analyze the relationship between Bordeaux concentration and the deposit remaining after run-off. The size of deposit remaining once the spray liquid has reached the leaf and started to run off is influenced by 1) the attraction of the spray particles for the sprayed surface, 2) the attraction of these particles for each other, and 3) the volume of run-off. If it can be assumed that the volume of run-off with increasing spray concentration is constant for a given spray time and foli-

age, then the amount of deposit remaining will be determined by the attraction of the particles for the leaf surface and each other. The assumption of constant volume run-off can be made for Bordeaux as the suspended particles do not noticeably change the surface tension of the fluid, within the range of concentrations used. If then the most important factors are the inter-attractions of spray particles and surface being sprayed, then the forces involved should be those operative in adsorption. The relationship between concentration of adsorbate and amount adsorbed by a unit of adsorbent has been investigated by Langmuir (15) who has proposed a mathematical equation for their relationship. At constant temperature pab = y + (y × pa), where "y" is the amount of material adsorbed per unit area, "p" is pressure or concentration, and "a" and "b" are constants. If the relation between Bordeaux deposition and the concentration of Bordeaux being used is principally determined by adsorption, then the curves obtained from the field data should be described by Langmuir's equation for the adsorption at constant temperature. By solving for constants[5] and interpolating new points, it may be demonstrated that the relation between concentration and initial retention of Bordeaux on both beans and celery (Fig. 1) is described by Langmuir's equation.[6]

When the data (Table 5, p. 18) of Hopperstead, Goodwin, and Kadow (11) are analyzed in the same way, it may be seen that Langmuir's adsorption equation likewise approximates the deposition of Bordeaux on apples. Unpublished data from J. G. Horsfall for Bordeaux deposition on potatoes also follows the Langmuir equation. This type of increase of deposition with increasing Bordeaux concentration is independent of crop, leaf measuring technique, or method of metal analysis. Whereas, the bean and celery leaves in the 1949 experiment were measured photoelectrically, Horsfall took his potato leaf samples on the basis of leaf weight, whereas Hopperstead et al used a punch to remove uniform discs of apple leaves. The deposits on the bean and celery leaves were analyzed spectrophotometrically, whereas both Horsfall and Hopperstead used the colorimetric method of Callan and Henderson (2) for their copper analyses. The bean and celery deposits are expressed as μgm. per sq. cm. of leaf surface, Horsfall's deposit data are expressed as the percentage of metal per unit weight of potato leaves, while Hopperstead presents his deposit data as μgm of copper per sq. in. of leaf surface.

What is the significance of this similarity of Bordeaux deposition to the adsorption equation? If it may be assumed that the leaf surface acts as an adsorbing surface (12) initial adherence is dependent, therefore, on contact between the leaf surface and the Bordeaux particles suspended in the spray droplets. All other factors being equal, the following may be postulated. At low concentrations the adsorptive surfaces have room for all the Bordeaux particles, and so adsorption varies directly with concentration. As spray concentration increases, however, there are fewer chances for all the spray particles to find an uncovered adsorptive spot. All those spray particles which are not held are lost in the run-off liquid. Eventually the adsorptive surface is completely saturated with Bordeaux particles so that at the higher concentrations of Bordeaux, the spray deposit increases very little, regardless of concentration increase. This phenomenon is dependent on run-off to remove the unadsorbed particles. Attraction of the spray particles for each other cannot be important in initial retention of Bordeaux, or this relationship would not exist.

Of the possible factors involved in holding particles to a surface, electrostatic and electrokinetic forces are of the most obvious importance. Wampler and Hoskins (23) could find no correlation between electrostatic charge and size of arsenical deposit. They sprayed lead arsenate on wax-coated glass plates. Because the surface being sprayed is moist, it is to be expected that electrostatic charges on spray particles would be rapidly lost on contact by grounding. Electrostatic forces are undoubtedly much more important in building up dust residues, where the particles are in contact with a dry leaf surface. Because the Bordeaux curves for initial retention indicated that adsorption is involved, electrokinetic forces must be operative. Work by Moore (19) and discussions by Hooker (10) and by Frear and Worthley (7) have suggested the role of electrokinetic forces in holding spray deposits to leaves.

To determine the charge of Bordeaux particles, a 4–2–100 Bordeaux mixture (pH 11.5) was tested cataphoretically in a Mattson cell using carbon electrodes and an E.M.F. of 45 volts, from 2 "B" batteries as the source of current. After 4 hours, the Bordeaux particles had moved up in the arm containing the negative electrode and down in the arm with the positive electrode. Bordeaux particles, therefore, were found to have a positive charge. As the moist leaf surface is negatively charged (19), the necessity for contact between leaf surface and Bordeaux particles for initial retention may well be explained on the basis of electrokinetic forces.

INITIAL RETENTION OF ZINEB.—There are 2 possibilities to explain the arithmetic increase of zineb deposits with concentration (Fig. 3). The first is that adsorption may be operative, but cannot be detected in the field data because none of the concentrations contained spray particles in sufficient number to approach saturation of the adsorptive surface. In other words, the concentrations which were used were not high enough to develop the curved portion of the adsorption isotherm. The second explanation may be that adsorption is not involved. Regardless of the explana-

[5] Constants "a" and "b," for Bordeaux deposition (Graph 1):

	a	*b*
On beans	0.275	6.37
On celery	0.440	4.70

[6] The author is indebted to Dr. A. E. Dimond for pointing out that the relation of Bordeaux deposition to concentration is described by Langmuir's adsorption isotherm.

tion, arithmetic relationships would exist only if the attraction of the spray particles for the leaf surface were equal to the attraction of the spray particles for each other.

To test whether or not adsorption is important in the initial retention of zineb, it became necessary to know the charge on the zineb particles. Using the apparatus previously described for Bordeaux, and using a concentration of 2 lb. per 100 gal. (pH 5.5), it was found that zineb particles are negatively charged. It becomes apparent that the electrokinetic conditions necessary for adsorption are not present in the case of zineb sprayed on leaf surfaces. Hence, the initial size of zineb deposit would be strictly proportional to the volume of spray remaining on the leaf surface and the concentration of the spray used. This would account for the arithmetic relation between initial retention and spray concentration.

Another point of difference between the initial retention of zineb and Bordeaux was their interactions with leaf surface. Celery leaves consistently retained more zineb deposit from the initial spray than did bean leaves. This was also true of the nabam mix. This difference was not apparent in the case of Bordeaux. In fact, there was slightly more Bordeaux deposited on beans than on celery. It was thought there might be some difference in spreadability of the 2 spray mixtures on the 2 leaf surfaces that might account for the difference in spray residue retention. The relation of area of spread to residue retention has been described and discussed by Woodman (27), O'Kane et al (20), Wilcoxon and Hartzell (25), and by Evans and Martin (3). Roughly, the greater the area of spread of the spray droplets, the more material is retained by the leaf surface.

One of the techniques for measuring ability of a liquid to spread on another liquid is to use the spreading coefficient described by Harkins and Feldman (8). Their formula is as follows: spreading coefficient = surface tension of liquid-air interface multiplied by (the cosine of the contact angle minus 1). This gives a negative number. The nearer the number is to zero the greater is the tendency of the liquid to spread on that particular surface. Adams and Jessop (1) showed that the spreading coefficient also applies to the spreading of a liquid on a solid. Although Harkins and Feldman (8) used the equilibrium contact angle in their formula, Evans and Martin (3) have suggested that the advancing contact angle would usually suffice for determining the tendency of a liquid to spread on a solid.

To detect differences in the spreading of Bordeaux and zineb formulations on celery and beans, suspensions of 8–4–100 Bordeaux and Dithane Z-78 at 4 lb. per 100 gal. were used. Leaf strips, 20 × 10 mm. in size, were placed right side up on the stage of a Bioscope microprojector. One drop each of Bordeaux, zineb suspension, and distilled water were touched down on the leaf strip from the tips of 0.1 cc. calibrated pipettes. The drops were 0.01 cc. in volume. The shadows of the drops, magnified 6 times, were projected on a ground glass, and the outlines of the contact angles were drawn on onion-skin paper. The angles were drawn within 3 minutes of the time the drop touched the leaf strips. Measurements were made on 10 strips of bean leaves and 10 strips of celery leaves. Analyses of variance were made on the sines of the contact angle.

On the upper surface of bean leaves, the contact angle for 8–4–100 Bordeaux was significantly different from those of 4-100 Dithane Z-78 and of water. The contact angle for Dithane Z-78 on beans did not differ significantly from that of water. There was no significant difference between the contact angles of the 3 liquids on the upper surface of celery leaves.

To use the spreading coefficient, the surface tensions of the liquids must also be known. The surface tension for Bordeaux had been found to be the same as for water. The surface tension of zineb suspensions were measured using the technique described for Bordeaux. The concentrations of zineb formulation tested were 1, 2, 4, and 8 lb. per 100 gal. None of the suspensions had surface tensions which were significantly different from that of water.

As the liquid-air surface tensions of all 3 liquids are the same, 72 dynes per cm^2 will be used in all spreading coefficients. On the upper surface of bean leaves, the spreading coefficient for Bordeaux was 72 $(0.250-1) = -54$. For zineb and for water it was 72 $(0.167 - 1) = -60$. On the upper surface of celery leaves, the average spreading coefficient for all 3 liquids was 72 $(0.286 - 1) = -51$. Hence, the ability of zineb droplets to spread on bean foliage is different from the ability of the droplets to spread on celery. Bordeaux droplets, however, would not spread a great deal more readily on celery than they would on beans. Actually the difference of interest is the ability of Bordeaux to spread significantly better than zineb and water on beans, in spite of having the same surface tension.

This phenomenon can be explained thermodynamically using the reasoning of Harkins and Feldman (8) in arriving at their spreading coefficient. The spreading coefficient is actually the work of adhesion (W_a) minus the work of cohesion (W_c). W_c is equal to twice the surface tension of the liquid. As the surface tensions are identical for Bordeaux, zineb and water, they may be ignored. W_a is the work done in pulling a column of liquid 1 sq. cm. in cross-section away from a solid surface. With W_c equal, the greater the W_a the more readily the liquid will spread on that surface. W_a is equal to the solid–air surface tension (T_{sv}) plus the liquid–air surface tension (T_{lv}) minus the solid-liquid surface tension (T_{sl}). In these experiments T_{sv} and T_{lv} are all identical, hence differences in amount of spread can be accounted for by differences in T_{sl}. In the case of Bordeaux, the adsorption of the suspended particles by the leaf surface would reduce T_{sl}, resulting in a larger W_a, and hence a greater area of spread. It is to be expected, there-

fore, that Bordeaux particles would tend to spread more readily on hard-to-wet leaves than would zineb.

To summarize, Bordeaux spreads on beans almost as readily as it does on celery, so that the initial retention of Bordeaux is about the same for both types of foliage. The spread of zineb formulation, however, is significantly less on beans than it is on celery, hence the initial retention of zineb on beans is distinctly less than it is on celery. These differences may be explained thermodynamically on the basis of adsorption phenomena.

WEATHERING OF RESIDUES.—After the deposits have dried, removal of the materials by weathering takes exactly opposite paths for Bordeaux and zineb. For Bordeaux, the larger the deposit, the smaller the percentage of that deposit lost by weathering (Fig. 2). The larger the zineb deposits, however, the greater is the percentage lost by weathering (Fig. 4).

By plotting the average deposits against the absolute amounts lost by each deposit, it is possible to determine the relative importance in weathering of particle–particle cohesion versus particle–leaf adhesion. The smaller the deposit, the greater the proportion of it as a monoparticulate layer in contact with the leaf surface. The larger the deposit, the smaller proportion of it will be monoparticulate. Of increasingly greater importance with increasing mass of residue will be the multiparticulate layers in contact only with other spray particles. If cohesion between particles is equal to adhesion of particles to leaf, then the proportionate amount lost by constant weathering will not change with the size of initial residue. A material of this sort will result in a straight line graph when size of deposit is plotted against amount of residue lost through weathering. By the same token, particles adhering more tightly to the leaf surface than they cohere to each other will produce a weathering curve which is positive concave upward. Particles cohering more tightly to each other than they adhere to the leaf surface would give a weathering curve which is positive convex upward. Fig. 5 shows the zineb weathering curves which are positive and concave upward, indicating that dried zineb particles do not stick together nearly so well as they do to the leaf surface. This is true regardless of the foliage. Zineb deposits on hairy bean foliage, however, resist weathering more than zineb on smooth celery foliage. The weathering curve for Bordeaux on celery (Fig. 6) is positive convex upward, indicating that the dried Bordeaux particles stick together more tightly than they do to the leaf surface. Bordeaux deposits on beans (Fig. 6), however, seem to follow an entirely different weathering pattern from Bordeaux on celery. Not only is the curve negative concave upward, but there is more actual material lost by the smaller deposits than there is from the larger. As the curve must go through the zero intercept, it is very likely discontinuous. It is possible that the smaller dried Bordeaux particles stick very poorly to bean leaves. As a result, most of the smaller deposits would be lost in their entirety by subsequent weathering. If this is true, then the multiparticulate layers of increasing importance in the larger deposits must in some way reduce the loss of the particles in contact with the leaf. Bordeaux forms a crust as it dries. This crust may actually overlayer and protect those particles in direct contact with the leaf surface. The larger the residues, the greater the number of multi-layered flakelets with protecting crusts. The bean leaf hairs also help to make larger Bordeaux deposits more resistant to weathering. As the Bordeaux residues increase in size, there are increasingly greater numbers of coalesced deposits which surround and stick to one or more leaf hairs. These hairs act as anchors for the flakelets of Bordeaux residue, and increase their resistance to weathering. This action would not be a factor in the weathering of zineb, where large flakelets are not formed. Large flakelets of zineb are not formed because 1) zineb particles do not stick tightly to each other, and 2) the actual initial deposits are much smaller than are those of Bordeaux.

This difference in weathering between the deposit of Bordeaux and zineb is probably related to the difference in the degree of hydration of the particles in the original spray suspensions. The particle to particle cohesion of the dried deposits of hydrophilic sols with highly hydrated particles, such as gelatin, clay, and agar, is well known. The dried deposits of hydrophobic suspensions, however, are easily broken up and weathered. One of the properties of hydrophilic suspensions is rapidly increasing viscosity with increasing concentration, whereas the viscosity of hydrophobic suspensions increase very little with concentration (24). To test the relative degree of hydration of Bordeaux and zineb, the viscosities of increasing concentrations of water suspensions of the 2 materials were tested in a #100 Ostwald viscosimeter in a thermostatically controlled water bath at 25° C. The fluid volume was 10 cc. and 4 readings were made at each concentration. All replicate flow-time readings checked within 0.2 second. The increase in relative viscosity with concentrations of the spray materials is shown in Fig. 7. The sharply rising curve for Bordeaux is typical of hydrophilic sols. The experiment was repeated using carbonated lime instead of fresh lime in order to test the effect of concentration load alone, without the formation of Bordeaux. In this case, there was only slight relative viscosity increase with concentration, as would be expected for a hydrophobic suspension. This demonstrated that the change in viscosity with increasing concentration of Bordeaux is actually caused by the hydration of the fresh Bordeaux particles. When zineb was tested in the viscosimeter, the resulting curve was typical of a hydrophobic suspension (Fig. 7). Bordeaux, then, is a hydrophilic suspension, whereas zineb is hydrophobic.

Martin (18) showed that when Bordeaux residues are dehydrated they lose their resistance to weathering. He found that continuous washing of the residues with

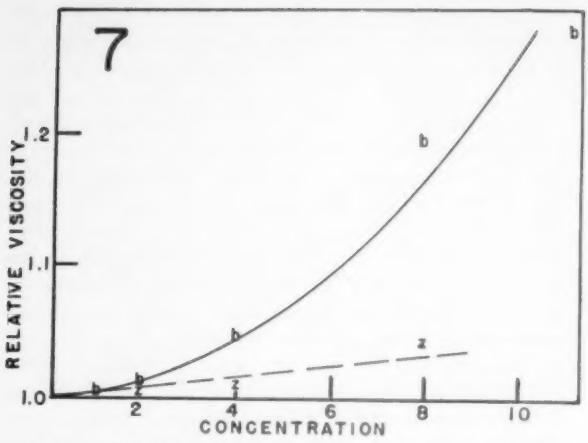


FIG. 7. Relation of concentration of Bordeaux and zineb suspensions to their relative viscosity. Concentrations expressed as lb. of $CuSO_4$ per 100 gal., and pounds of zineb formulation per 100 gal. Bordeaux indicated by "b"'s and solid line. Zineb indicated by "z"'s and broken line.

CO_2-free water changed the hydrated blue Bordeaux to the brown, non-adhesive, cupric oxide. Apparently the absorbed sulphate and excess calcium hydroxide are removed by dehydration with CO_2-free water. When CO_2 is present, however, some obscure reaction takes place which prevents dehydration by washing, and preserves the Bordeaux as the blue, hydrated, adhesive deposit.

APPLICATIONS.—What has been learned from the analysis of the deposit and residue data may be used for improving the efficiency of field spraying. Bordeaux in general will weather more slowly than zineb. The rate of weathering of either material, however, will be determined by the type of foliage to which it has been applied. Hairy foliage will lose deposits more slowly than smooth leaved plants. Hence hairy plants need not be sprayed quite so often as smooth plants. Because the leaf hairs hinder the passage of the spray droplets to the leaf surface, it is probably advisable to alter the spraying technique when spraying hairy foliage so that a greater number of droplets will drive through to the leaf surface. This can be done by increasing the velocity of the droplets, or increasing the number of droplets having diameters of 50–100 μ.

Bean foliage not only loses less deposit by weathering, but also picks up less material from succeeding sprays. For efficiency, it may be well to reduce the concentrations of spray liquid being applied to hairy surfaces which have already received at least 1 spray application.

The relation of Bordeaux concentration to deposition by standard hydraulic spraying follows a law of diminishing returns. Increasing Bordeaux concentrations much beyond 6–3–100 or 8–4–100 would, therefore, be very inefficient. Most of the Bordeaux particles in excess of these concentrations probably fall to the ground.

Adsorption of spray particles to a leaf surface is an aid in building up deposition. As the moist leaf is negatively charged, it can be predicted that cationic compounds would have more tenacity than anionic materials, other things being equal. This knowledge can be useful in searching for protectant fungicides.

In addition to using the data to improve spraying practice, certain of the theories proposed from studying the data aid in the understanding of build-up of deposit, and the redistribution of dried deposits. It has long been puzzling why the low-pressure, low-gallonage apparatus of the early days of plant protection gave such good control of certain diseases such as apple scab. In spite of the tremendous technical strides in recent years, apple scab control with sulfur today is not much better than it was in 1915. High pressure and high gallonage avails us very little. Marsh et al (17) reported on the effective use of copper fungicides applied with a watering can. Recently, J. D. Wilson (26) reported good results with low gallonage, low-pressure hydraulic application of concentrated sprays. These experiments probably owe their success to the production of heavier deposits through a minimum of run-off. Although initial coverage was undoubtedly poor in both cases, the materials were probably redistributed by rain to give adequate surface protection.

In his discussion of redistribution Horsfall (12) poses the problem of how it is that a rolling rain drop can pick the spray material from a sprayed leaf and deposit the material on a lower unsprayed leaf. The adsorption theory furnishes an explanation for this phenomenon. The rain drop picks off the top layers of the spray deposit as the drop rolls across the sprayed leaf. There is little tendency for redeposition except as the drop may cross an unsprayed and therefore receptive adsorbing area. If the drop carrying spray material falls onto a lower unsprayed leaf, the material is redistributed to the fresh adsorbing surface.

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MODIFIED LYOPHIL METHOD FOR PRESERVATION OF PYTHIUM SPECIES [1]

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SUMMARY

For the first time, *Pythium* species have been successfully lyophilized. This success is attributed to the use of organic plant parts as the suspending media. All other liquid and semi-liquid suspending media tested failed to give satisfactory results.

The oogonium-producing isolates of *P. acanthicum* and *P. irregulare* survived in all cases while those of *P. paroecandrum* and *P. debaryanum* survived occasionally. The sporangium- and mycelium-producing isolates tested did not survive.

Rehydration facilitated more rapid development of lyophilized *Pythium* species.

Lyophilization has become one of the most promising methods for prolonged preservation of fungi. Raper and Alexander (3) in 1945 reported the lyophilization of representative fungi from the main groups, with special emphasis on those used in fermentation, and obtained good results with all species producing abundant small spores. Fungi having large spores such as *Conidiobolus* and *Entomophthora* did not survive. Later *Pythium* and *Achyla*, producing more specialized oospores, gave negative results (1). The lyophilization of these fungi involved the use of blood serum as suspending medium. Other workers have found it necessary to use special types of suspending media to obtain maximum survival (2, 5).

In this work the lyophil process has been modified to include fungi having large, specialized spores. This has necessitated the use of a different suspending medium from those previously incorporated.

Materials and methods.—Beef blood serum was first tried as a suspending medium for hyphae and oogonia which were removed from a culture with a flamed transfer needle and placed in the medium contained in lyophil tubes. Next, agar blocks containing hyphae and oogonia were cut out and placed in lyophil tubes for processing. Then a gelatin medium was used to culture and suspend the *Pythium* isolates. Finally, oat straws were evaluated as a possible suspending medium.

Healthy, unbroken Clinton oat straws 3 in. long were impregnated with water under evacuation, and

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steam sterilized for 45 minutes at 15 lb. pressure. The water was decanted and straws were placed aseptically on 2-day-old carrot agar[2] cultures of *Pythium* contained in 9-cm. petri plates. These straws remained in the petri plates until thoroughly colonized and until oogonia were formed profusely in the agar. In order to assure uniform colonization of the straw and uniform oogonial production in and on the straw, the latter were then transferred to freshly poured petri plates and left until oogonia were produced to the extremity of the agar. Straws were removed aseptically, cut into 1 cm. pieces and placed in lyophil tubes.

The processing was done by 2 main methods. In 1 case, the material was frozen at −45°C. by immersion in a dry ice-acetone bath and then the bath temperature was either allowed to rise slowly over a temperature range of −45°C. to room temperature, or brought up immediately to −10° or −20°C. for a subsequent drying period of about 3 hours. Finally, the tubes were exposed to room temperature for about 30 minutes before sealing under vacuum with a cross-fire gas-oxygen torch. In the other method, the material was not pre-frozen and the lyophil tubes were exposed at room temperature throughout the entire drying period of 3 hours. All sealed tubes were then stored at room temperature and the straws were removed at various intervals for viability tests. In these tests the tubes were opened, lightly plugged with cotton, and placed in a saturated moist chamber at room temperature for various time intervals, unless otherwise stated. After rehydration, the colonized straws were placed on the surface of carrot agar and observed for *Pythium* growth.

Results.—Suspending media of blood serum, agar blocks, and gelatin failed to yield viable *Pythium* cultures following the drying process. However, preliminary investigations incorporating straws as a suspending medium for *P. acanthicum* showed promise.

Of a number of different processing methods used with *P. acanthicum* all trials with straws were successful, but the lots dried at −10°C., at gradually rising temperature between −45° and −10°C., and at room temperatures required less time to initiate growth. For these lots, mycelium was first observed growing from the straws 1–4 days after plating on carrot agar. Samples dried at −20°C. often required a week or more to initiate growth. Since the processing method not utilizing a low temperature bath was more convenient, this method was used for processing several other *Pythium* species.

Three types of development were represented among the 9 *Pythium* species lyophilized. These included the production of oogonia by *P. acanthicum* Drechsl., *P. irregulare* Buis., *P. paroecandrum* Drechsl., *P. debaryanum* Hesse and *P. aphanidermatum* (Edson) Fitzp.; of sporangia by *P. rostratum* Butl., *P. splendens* Braun and *P. arrhenomanes* Drechsl.; and of mycelial growth by *P. graminicolum* Subram.

To obtain rapid development following the lyophil process, the lyophilized straws containing *Pythium* were rehydrated in a saturated moist chamber before plating onto agar. Mycelial growth from rehydrated, colonized straws occurred within 1–4 days, while similar growth from non-rehydrated, colonized straws often required a week, and in a few cases no growth was observed. Rehydration periods between 12 and 60 hours were equally effective in obtaining positive results, but after 72 hours in the moist chamber the straws often become contaminated with bacteria.

All tests of *P. acanthicum* and *P. irregulare* gave positive results; the former was tested 22 times and the latter 7 times. *P. paroecandrum* and *P. debaryanum* were each tested 11 times and 3 samples of each species gave positive results. Following 48 hours of rehydration, growth of *P. acanthicum* and *P. irregulare* usually occurred in 1–3 days, while growth of *P. paroecandrum* and *P. debaryanum* occurred after a week or longer. Another oogonia-producing species, *P. aphanidermatum*, as well as the 3 sporangia-producing species and the mycelium-producing form, did not survive lyophilization. In all cases where lyophilization was successful, the oogonia produced were typical of corresponding structures in nonlyophilized cultures.

Pythium acanthicum has remained viable over a 14-month storage period, and there has been no evidence of viability decline.

In addition to *P. acanthicum* and *P. irregulare*, a lyophilized zygospore-producing *Zygorhynchus* sp. gave positive results.

Discussion.—The lyophil process as used by Raper's group (1) was unsuccessful for those forms of fungi producing large or specialized spores and for those producing only sporangia and mycelium. In the present work, the suspending medium was found to be the most critical factor for lyophilization of large, specialized spores. The reason for success obtained when using organic plant parts as a suspending medium is not definitely known. However, the straws contained less water than the other media and had a larger surface, which may have permitted faster freezing and drying or allowed less mechanical damage to the cells during freezing. The straws may also have been a more favorable medium for the rehydration stage which has been shown to be an important factor in the case of *Puccinia* uredospores (4).

P. acanthicum and *P. irregulare*, the only 2 forms tested having extensive surface area due to the projections on the oogonial wall, always gave positive results. In contrast, *P. paroecandrum* and *P. debaryanum*, both producing spherical, smooth-walled oogonia, survived lyophilization only occasionally. To date, *P. acanthicum* has been stored at room temperature for 14 months with no viability loss. It seems likely that these cultures should be viable for years, since Raper's group (1) found that forms which survived lyophilization were still viable after 7 years' storage.

[2] Prepared from 18 gm. "Difco" Agar, No. B140, 750 ml. of distilled water and 250 ml. of aqueous carrot extract prepared by autoclaving 30 gm. diced carrots in 250 ml. of distilled water for 30 minutes at 15 pounds pressure.

Negative results with *P. aphanidermatum* were attributed to aborted or disintegrated oogonia. The 3 sporangium-producing species and the mycelial form failed to survive, as has been previously noted (1).

The present work suggests that the use of non-liquid suspending media and varying the conditions of rehydration and lyophilization might result in successful processing of other fungi which have not heretofore survived lyophilization.

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RHIZOCTONIA AERIAL BLIGHT OF SOYBEANS IN LOUISIANA[1]

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SUMMARY

For 3 consecutive years, 1950-1952, Rhizoctonia aerial blight caused by *Pellicularia filamentosa* (Pat.) Rogers (syn. *Corticium microsclerotia* [Matz] Weber) was observed in soybean nurseries and nearby test fields at Baton Rouge, Louisiana. The causal pathogen was consistently associated with the symptoms of leaf spots, leaf blight, and necrosis of other plant parts. The disease first appeared during the summer and persisted during the fall. In 1951 and 1952 microsclerotia were produced abundantly under field conditions. All *Rhizoctonia* isolates tested were pathogenic on soybeans under controlled conditions. Varietal differences in susceptibility were observed.

A rather striking and unusual foliage disease was observed in the U. S. Department of Agriculture regional soybean nursery planting at Baton Rouge, Louisiana, and nearby test plots of the Crops and Soils Department, Louisiana Agricultural Experiment Station, in 1950, 1951 and 1952. Investigation showed that the disease was caused by a fungus originally described as *Rhizoctonia microsclerotia* Matz (4), later called *Corticium microsclerotia* (Matz) Weber (10), and finally listed as a synonym of *Pellicularia filamentosa* (Pat.) Rogers (5). A preliminary report of the disease was published in 1952 (1). Studies on the fungi causing leaf or aerial blights in the southern states have been made by Weber (10), Tims and Bonner (8), Tims and Mills (9), Ryker (6), Ryker and Exner (7) and others. Certain aspects of the disease will not be emphasized since they have been covered adequately by Weber (10) in his work with the same fungus as the cause of web blight of snapbeans in Florida. Although *Rhizoctonia* has been reported as the cause of root, stem or seedling diseases of soybean (2), aerial leaf blight has not been studied insofar as the writers are aware. However, Drs. S. G. Lehman and Howard W. Johnson observed limited Rhizoctonia aerial infection on soybeans in North Carolina in 1944 and made studies which were not reported.[4]

Symptoms.—Leaf spots, leaf blight, and defoliation were symptoms of the disease observed in the field. The necrotic areas varied in size from small spots approximately 2 mm. in diameter to those covering the entire soybean leaflet. Leaf symptoms are illustrated in Fig. 1. Typically the infected area involved the lower third of 1 or more of the 3 leaflets. The necrotic areas varied in shape from circular to irregular with indefinite to distinct reddish brown margins. Color of lesions varied from greenish brown to reddish brown but typically they were tan or brown. The symptoms varied somewhat on the different varieties. Brown lesions were frequently observed on the petioles and stems. When the young pods were attacked they developed brown necrotic areas or a complete necrosis. The disease was not limited to leaves near the soil surface but much of the infection occurred on leaves 2 ft. or more above ground. Infection was generally not uniform within a field or variety plot; instead the disease was present in rather definite areas consisting of a few plants along a row or adjacent rows. Frequently plants in limited areas showed 50 per cent to nearly complete defoliation.

Generally the cobweb-like mycelium of *Rhizoctonia* was observed on or around the necrotic areas, particularly between adjacent infected leaflets growing in close proximity. In 1951 and 1952 microsclerotia

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[4] Letter dated Oct. 31, 1950 from Dr. G. S. Lehman, North Carolina State College, Raleigh, North Carolina.
Letter dated Oct. 4, 1950, from Dr. Howard W. Johnson, Division of Forage Crops and Diseases, U. S. Dept. of Agriculture, Stoneville, Mississippi.

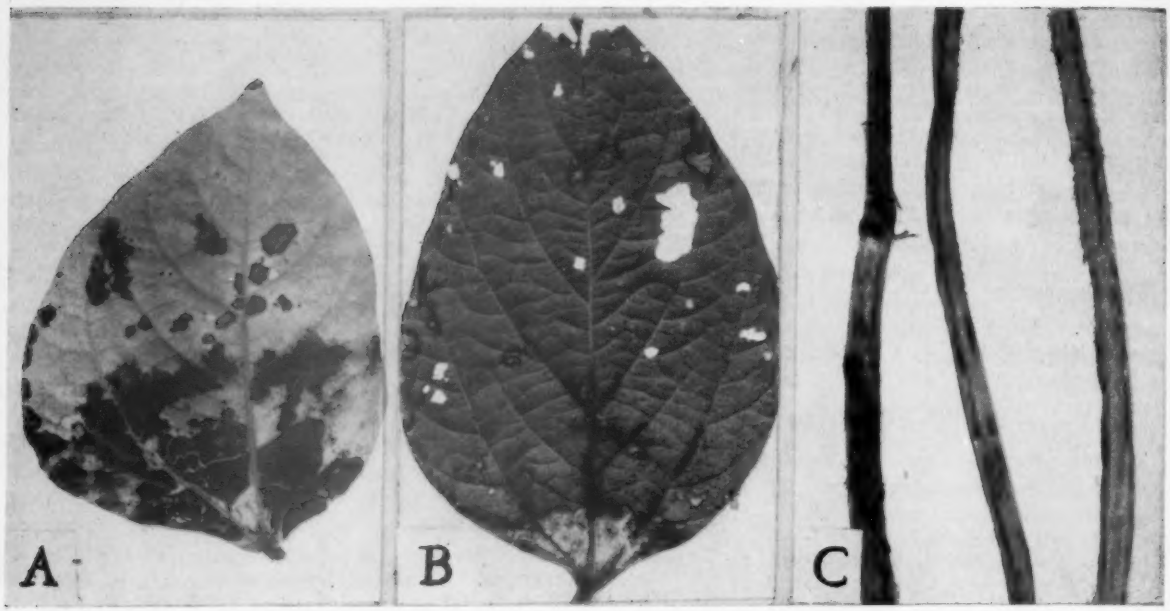

FIG. 1. A) Rhizoctonia blight symptoms on *Dolichos* sp.; B) Symptoms on soybean selection La. 48-275; C) microsclerotia on soybean petioles.

were produced in abundance, chiefly along the petioles of infected leaves (Fig. 1). Few sclerotia were evident on the surface of invaded leaf areas. In 1950 no sclerotia were observed. Occasionally large sclerotia were observed on soybean plants in addition to the typical microsclerotia. Since microscopic examination of the microsclerotia showed that they were oval to irregular in outline rather than circular, the longest dimension was measured. The average size of 20 single microsclerotia was 159.9μ (range, 99.9–166.5μ). Sclerotial aggregates consisting of chains or masses of microsclerotia were frequently observed. These were generally linear in shape and measured up to 330μ in length. A basidial stage was observed but not studied.

SEASONAL DEVELOPMENT AND DISTRIBUTION. — Although the disease may have been present earlier, Rhizoctonia aerial blight was first observed at Baton Rouge in mid-July 1950, late July in 1951 and on July 24 in 1952. High temperature and high humidity were considered necessary for disease development. Spread and development were limited by dry weather or low temperature but the disease persisted in infected fields or plots until frost or until the beans matured. The disease was not observed in similar soybean nurseries at 2 locations in north Louisiana, Northeast Louisiana Agricultural Experiment Station, St. Joseph, and Red River Valley Experiment Station, Bossier City. However, a field of Biloxi soybeans near Hammond, Louisiana, showed light infection in 1950.

ISOLATIONS AND PATHOGENICITY TESTS.—In 1950 and 1951, *Rhizoctonia* was consistently isolated from infected leaf, petiole, stem and pod tissues. In 1951 isolates were also obtained from single microsclerotia. Although the various isolates showed a certain amount of variation in cultural appearance on potato-dextrose agar, nearly all of them fell into a fairly distinct cultural type when obtained from tissues showing a typical infection collected at a distance of 12 in. or more from the soil surface. Isolates of other cultural types were obtained by isolating from diseased basal leaves growing near the soil surface, basal stem tissues, roots or damped-off seedlings. Most of such isolates were considered typical of *R. solani*, the common soil or damping-off type encountered in blighted seedlings or stem rots of various plants. A few isolates of the "sasakii" type were obtained from small plants growing among infected grasses.

The various *Rhizoctonia* isolates from diseased soybean leaves produced typical symptoms when used to inoculate the aerial plant parts of soybeans in the greenhouse under controlled conditions. As in the field, symptoms varied from small leaf spots to necrosis of entire leaflets or other plant parts. Symptoms appeared within 24 hours on the leaves of inoculated plants held in a moist chamber at about 30° C. Microsclerotia were not produced on the inoculated plants. Isolates from soybean leaves, stems and pods, snapbeans, and tie vine (*Jacquemontia tamnifolia*) were all pathogenic on the leaves of soybean selection La. 48-275. Isolates of the "solani" and "sasakii" types from soybeans and other plants also proved to be pathogenic on soybean foliage. The *R. solani* type isolates added to steamed soil were more pathogenic than those of the microsclerotia type when a reduction in emergence was used as the basis for comparison.

VARIETAL SUSCEPTIBILITY. — Dortchsoy 31 was conspicuous among the varieties and selections included

TABLE 1.—*Rhizoctonia disease ratings of some soybean varieties included in the U.S.D.A. regional soybean nurseries*

Variety	Average Disease Rating 1950	1951	1952
Acadian	0.0[a]	0.0	0.2
Roanoke	0.9	0.1	1.0
Improved Pelican	0.0	0.0	0.2
N46-2881	0.0	0.1	0.7
Dortchsoy 31	2.0	2.3	2.2
Ogden	1.7	0.5	2.5
Hale Ogden	1.4	0.6	3.0
Majos	1.7	0.8	1.5
La. 48-275	1.2	0.8	—

[a] Rating scale: 0-5. Average for 4 replications. 0 = resistant; 5 = susceptible.

in maturity group VII as a result of its susceptibility to Rhizoctonia aerial blight for 3 consecutive years, 1950-1952 inclusive. While Dortchsoy 31, Ogden, and other varieties were rather consistently rated as susceptible, others were considered resistant on the basis of disease ratings. Disease ratings for a few varieties, susceptible and resistant, are in Table 1.

Of the 8 soybean varieties or selections listed in Table 1, Acadian, Roanoke, Improved Pelican, and N46-2881 were considered as having some resistance under field conditions in comparison with the other 5 varieties.

A few soybean varieties were grown in the greenhouse and inoculated in a varietal resistance test. Although all varieties were susceptible, (Table 2), some exhibited slight resistance.

HOST RANGE.—Among cultivated plants growing near the soybean test plots, cowpeas, crotalaria, and *Dolichos* sp. were susceptible to the *Rhizoctonia* strain producing microsclerotia on the host plant. In addition the same disease was observed on snapbeans near Ponchatoula, Louisiana, in 1950, but this was not the first occurrence of the disease on snapbeans in the state. Among the grass or weed hosts were *Jacquemontia tamnifolia* (tie vine), *Cyperus* sp. (Sedge), *Mollago verticillata* (carpet weed), *Digitaria ischaemum* (smooth crab grass), *Echinochloa coloma* (jungle rice) and *Eleusine indica* (goose grass). The fungus was probably present on other weeds, grasses or cultivated plants.

In artificial inoculation studies, snapbeans, cowpeas, lima beans, trefoil, Korean Lespedeza, tall fescue, fig, and tung were found to be susceptible to isolates from soybean. Weber (10) found a number of other plants to be susceptible to a similar fungus.

DISCUSSION.—The *Rhizoctonia* fungus that caused foliage or aerial blight of soybeans at Baton Rouge, Louisiana, appeared to be the same as that studied by Weber (10) as the cause of web blight of snapbeans in Florida. Although the species name of *R. microsclerotia* used by Matz (4) and Weber (10) is no longer accepted, a pathogen persists in Louisiana which causes a rather distinct foliage disease of various plants under conditions of high humidity and temperature with the production of abundant microsclerotia on the host plants. Unpublished studies by the

TABLE 2.—*Greenhouse test of soybean varietal resistance to Rhizoctonia aerial blight*

Variety	Average Disease Rating Control	Inoculated
OK-710	0.0[a]	4.0
Ogden	0.0	4.4
Majos	0.0	4.2
Roanoke	0.0	2.8
Acadian	0.0	2.8
Mamotan 6640	0.0	2.6

[a] Rating scale: 0-5. Four replications.

senior writer and the work of Exner (3), Ryker and Exner (7) and Tims and Bonner (8) on *Rhizoctonia* diseases of plants in Louisiana indicate that the fungus studied by Matz (4) and Weber (10) under the name of *R. microsclerotia* represents one of a group of rather distinct types, species, or strains presently grouped under the binomial *Pellicularia filamentosa.*

Although no sclerotia were observed on the diseased soybean plants in 1950, the isolates obtained were similar to those made in 1951 from single microsclerotia and from infected leaf tissue associated with microsclerotia. Since isolates of the *R. solani* type were also found capable of causing leaf infection under controlled conditions, various types of *Rhizoctonia* may be encountered on soybeans under conditions favoring aerial infection.

Although Rhizoctonia aerial blight has not been of economic importance on soybeans in Louisiana, the disease was more serious on certain varieties, such as Ogden, than other foliage diseases at Baton Rouge. Since high humidity and high temperature are essential for disease development on the basis of field observation and greenhouse studies, the disease can be considered of potential importance only in geographical areas having environmental conditions similar to those in south Louisiana. Thus, the disease cannot be considered of potential importance in the major producing areas of the United States.

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PHYTOPATHOLOGICAL NOTES

Insect Transmission of Western-X-Little-Cherry Virus.[1] Mervin W. Nielson and Laurence S. Jones.[2] Wolfe, Anthon, and Jones[3] first reported the transmission of western X-disease virus of peach by the geminate leafhopper, *Colladonus geminatus* (Van D.), followed by confirmation of the transmission by Wolfe, Anthon, Kaloostian, and Jones.[4] Anthon and Wolfe[5] reported 3 additional leafhopper vectors of western X-disease virus of peach, namely: *Scaphytopius acutus* (Say), *Fieberiella florii* (Stal), and *Keonolla confluens* (Uhler). Kaloostian and Nielson[6] demonstrated the transmission of western X-disease virus by the geminate leafhopper to peach from diseased peach, from sweet and sour cherry, and from choke cherry. Now, for the first time transmission of western X-disease virus from cherry to cherry by insects has been demonstrated.

In 1951, at The Dalles, Oregon, vector transmission tests were conducted with nursery trees in a plot isolated from commercial cherry orchards, to complete the gap in the insect transmission cycle from cherry infected with western-X-little-cherry to healthy cherry. Montmorency cherry on Mahaleb rootstock was selected as the test plant, since this combination has given a distinct foliage symptom following bud inoculations. J. H. Hale peach was also used as a test plant. Several hundred geminate leafhoppers were collected from alfalfa, and placed in sleeve cages on the following 3 types of source plants: western-X-diseased peach, western-X-little-cherry-diseased sour cherry, and western-X-little-cherry-diseased sweet cherry. After they had fed for the desired length of time, half the leafhoppers of each group were caged on the healthy sour cherry trees and the other half on the peach trees. The leafhoppers were caged on alternate trees, and the remaining adjacent trees were left as checks. After the insects had remained on the test trees for the desired length of time they were recollected for identification.[7]

Table 1.—*Summary of transmission tests of western X-disease virus to sour cherry and peach*

Source of Inoculum	Test Tree	Number of Tests	Number of Transmissions
Sweet cherry	Mont. cherry	10	3
Sweet cherry	Peach	5	1
Sour Cherry	Mont. cherry	10	4
Sour Cherry	Peach	7	2
Peach	Mont. cherry	9	0
Peach	Peach	8	0

The 49 tests in the first series of experiments are summarized in Table 1.

On May 22, 1952, approximately 1 year after the leafhoppers were put on the test trees, 7 Montmorency cherry trees had developed typical foliage symptoms of western X-little-cherry disease. Four of these infections resulted from sour cherry inoculum and the remainder from sweet cherry inoculum. An impressive example of transmission continuity was shown when 4 consecutive test trees developed disease symptoms while the 4 alternate or check trees remained normal, and were growing vigorously. One case of transmission was obtained on peach with leafhoppers of the same lot that transmitted western-X-little-cherry virus to Montmorency cherry. No apparent transmission was obtained from peach to cherry in this particular effort.

For virus acquisition the leafhoppers were permitted a feeding period of 37–46 days on trees infected with western-X-little cherry virus of cherry and western X-disease of peach. For the transmission feeding period the insects were given from 116 to 126 days on the healthy test trees. Symptoms appeared in test trees approximately a year after insects were caged upon them. Thirty-five per cent transmission to Montmorency trees was obtained with the leafhoppers that had fed on diseased sweet or sour cherry trees. In each test more than 25 insects were caged; both nymphs and adults were represented.

Symptoms on the experimentally infected Montmorency cherry trees were exhibited first by the inward cupping, rolling, and yellowing of leaves (Fig.

[1] Approved for publication as Technical Paper No. 809 by the Director of the Oregon Agricultural Experiment Station and as Manuscript No. BEPQ-Ms 3167 by the Chief of the Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture. Contribution of the Mid-Columbia Branch Experiment Station of OAES and of the Division of Fruit Insect Investigations of BEPQ.

[2] M. W. Nielson and L. S. Jones represented the Bureau of Entomology and Plant Quarantine. J. A. Milbrath and H. J. O'Reilly, plant pathologists in the Oregon Agricultural Experiment Station, gave guidance in all pathological considerations, including diagnosis of disease, test-plant maintenance, and inoculum selection.

[3] Wolfe, H. R., E. W. Anthon, and L. S. Jones. 1950. Transmission of western X-disease of peaches by the leafhopper, Colladonus geminatus (Van D.) Phytopathology 40: 971.

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[7] Appreciation is expressed to D. A. Young, Bureau of Entomology and Plant Quarantine, for the determinations of the insect specimens.

FIG. 1. Montmorency cherry: A) Normal branch from healthy check tree; B) Branch from tree with western-X-little-cherry, showing chlorotic, rolled leaves—infected as a result of feedings of viruliferous leafhoppers, *Colladonus geminatus* (Van D.)

1.). Later, the tips and margins of leaves became necrotic and the entire tree stunted. Four trees that showed these symptoms died before August 15, 1952. Symptoms were identical with those obtained when Montmorency on Mahaleb rootstock was inoculated by buds from trees infected with western-X-little-cherry virus.

As soon as they were recognized in early summer, 1952, each of these cases of transmission into cherry by leafhoppers was indexed into peach by bud inoculation. In the spring of 1953, at least 2 of these indexings had produced western X-disease in the peach.—Oregon Agricultural Experiment Station and U.S.D.A., Agr. Res. Adm., Bureau of Entomology and Plant Quarantine, Corvallis, Oregon.

Yellow-net Virus Disease of Tomato. EDWARD S. SYLVESTER. A yellow-net virus disease of tomato was first obtained from Dr. James T. Barrett[1] in the fall of 1946. Since that time the disease has been found to occur sporadically in tomato plantings at Berkeley, and in 2 instances in fields at Salinas, California.

The symptoms expressed in all tomato varieties tested, including *Lycopersicon esculentum* Mill., and *L. pimpinellifolium* Mill., are a pronounced yellow chlorosis of the veins and veinlets of infected leaflets (Fig. 1). Usually, in initial phases of the disease, the symptoms are bright coloration on new growth, but in all observed instances of infection, both in the field and greenhouse, symptoms became less and less noticeable as the infection aged. Normally, in a month or 6 weeks after onset, the vein and veinlet chlorosis faded until it was no longer evident. There is no apparent stunting or malformation of infected plants, and the set of fruit is normal. Seed from diseased plants when planted produced normal seedlings, and samples of these plants were susceptible to infection when inoculated by infective insects. Consequently the seedlings presumably were originally disease-free.

The incubation period of the disease in the Marglobe variety of tomato ranged from 11 to 31 days with a mean of 19.7 days.

The only aphid species that has been tested in replicated series is the green peach aphid, *Myzus persicae* (Sulzer), and this species transmitted the virus with a low level of efficiency. Approximately 17 per cent transmission was obtained in 217 trials using 10 aphids per plant. No standardization of acquisition or test feeding period was made. The transmission cycle can be completed within a 72-hour period, using a 24-hour acquisition feeding and a 48-hour test feeding period, and the aphids can retain the ability to transmit the virus for several days. Using groups of 10 aphids per plant and transferring infective insects daily to young tomato seedlings, infections were obtained on the fourth day out of a possible 7 days. In trials where infective insects (24-hour acquisition feeding on a diseased tomato) were transferred to healthy sugar beet plants for periods of 3, 5, 6, 7, 8, and 9 days, before being fed for 48 hours on a healthy tomato seedling, insect retention of the virus for 9 days was indicated.

Limited host range tests, including *Nicotiana glutinosa* L., potato, sugar beets, and cultivated geum (probably *Geum chiloense* Balb.), failed to produce symptoms.

Because of the resemblance of symptoms and vector

FIG. 1. Bright yellow chlorosis of the veins and veinlets, symptoms of the yellow-net virus disease on experimentally infected tomato, var. Marglobe.

[1] Professor of Plant Pathology, Emeritus, University of California, Berkeley, California.

relationships between tomato yellow-net virus and beet yellow-net virus,[2,3] reciprocal transmission tests with infective insects and both viruses and both hosts have been made. In no instance has inoculation with the tomato yellow-net virus induced infection in beets, nor has inoculation with the beet yellow-net virus produced infection in tomatoes. While it is possible that the 2 viruses are related, it is probably best, on the basis of present knowledge, to consider them distinct. —University of California, Berkeley 4, California.

[2] Roland, G. 1948. Sur une virose nouvelle de la betterave: La jaumisse des nervures. Parasitica 4: 152-154.

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LISTING OF BOOKS

PHYTOPATHOLOGY will, from time to time as space becomes available, list titles of books on phytopathological subjects. A brief description of the contents of the books will be included.

RODGERS, A. D. 3rd. 1952. **Erwin Frink Smith.** American Philosophical Society, Philadelphia. 675 p. price $5.00.

This is a story of the life and work of Erwin F. Smith and of his associations with other scientists of his time. It depicts the early beginnings and development of plant bacteriology in this country and abroad and touches upon various other phases of plant pathology, especially as it developed in the United States Department of Agriculture.

MATSUMOTO, T. 1952. **Monograph of sugarcane diseases in Taiwan.** Taipei, Tawai, China. 61 p. illus.

Following a key to diseases occurring on sugar cane, the author lists and describes 38 diseases and their causal agents. He includes observations on distribution, prevalence, and varietal susceptibility. Addenda describe various deficiency diseases. Fifteen plates, many in color, complete the volume.

SATTAR, ABDUS AND ABDUL HAFIZ. 1952. **Researches on plant diseases of the Punjab.** Pakistan Association for the Advancement of Science, University Institute of Chemistry, Lahore, Pakistan. 158 pp. price Rs. 12.

The first scientific monograph published by the Pakistan AAS represents the results of 20 years' research on the more important plant diseases in the Punjab. Scaled to the needs and limitations of both farmers and students, it gives simple, practical methods for control of diseases of crop plants and is well fortified with illustrations, tables, graphs, and references.

SCHRADER, FRANZ. 1953. **Mitosis; the movement of chromosomes in cell division.** Ed. 2. Columbia University Press, New York. 170 p. Price $4.00.

This second edition follows the general outline of the first but attempts a reorientation of viewpoint regarding the movement of chromosomes in cell division, in line with progress in the field since 1944. Beginning with a generalization of the process of mitosis, the book then discusses current theories and concepts and some related problems. An extensive bibliography in included.

FISCHER, G. W. 1953. **Manual of the North American smut fungi.** The Ronald Press Company, New York. 343 p. price $8.75.

This manual was prepared as a means by which anyone with some mycological background can identify any species of smut fungus known to occur in North America. It contains a combination host genus index and key to smut species and, in addition to the descriptions of species, a key to the genera of smut fungi.

BRAUN, H. AND E. RIEHM. 1953. **Krankheiten und Schadlinge der Kulturpflanzen und ihre Bekampfung.** Ed. 7. Paul Parey, Lindenstr. 44-47, Berlin SW 68. 339 pp.

The first edition was prepared by Riehm in 1910. In the present edition are discussions of diseases and insect pests of cereals, potatoes, sugar beets, carrots, clover, Lupine, soybean, alfalfa, serradella, beans, peas, crucifers, tomato, onion, asparagus, cucurbits, rape, flax, poppy, tobacco, hops, tree and bush fruits, and grapes, with a final chapter on organisms that attack various crops. The parts pertaining to symptoms, causal organisms, and life histories are valuable to both entomologists and plant pathologists, but the control measures are based on a lower economic level than is generally found in America.

WALLACE, T. AND R. W. MARSH, ed. 1953. **Science and fruit.** University of Bristol, Bristol, England. 308 p. price $4.50.

A jubilee volume commemorating the work of the Long Ashton Research Station, University of Bristol, since its foundation in 1903. Of interest to phytopathologists are 6 papers dealing with pests of apple, apple and pear scab, and insecticides and fungicides.

GAÜMANN, E. A. 1953. **The Fungi** (Translation by F. L. Wynd) Hafner Publishing Co., New York. 420 p. Price $10.00.

The morphological and developmental features of the fungi are described with emphasis on the natural relationships. There are over 400 figures and an extensive bibliography.